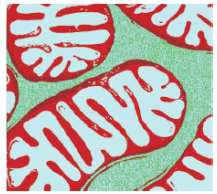
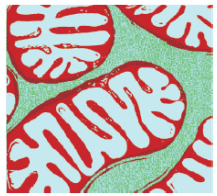
Nutrient Sensing, Acetylation, Mitochondrial Quality Control and Pathology



Michael N. Sack

Cardiovascular and Pulmonary Branch
National Heart, Lung and Blood Institute

NIH National Heart, Lung, and Blood Institute



Talk Outline

- Caloric Load, Sirt3 and the Regulation of Protein Acetylation
- Fasting and Tylenol Liver Toxicity

- Sirtuin Biology and Mitochondrial Quality Control
- The Role of Fasting and Sirt3 on NLRP3 Inflammasome Biology

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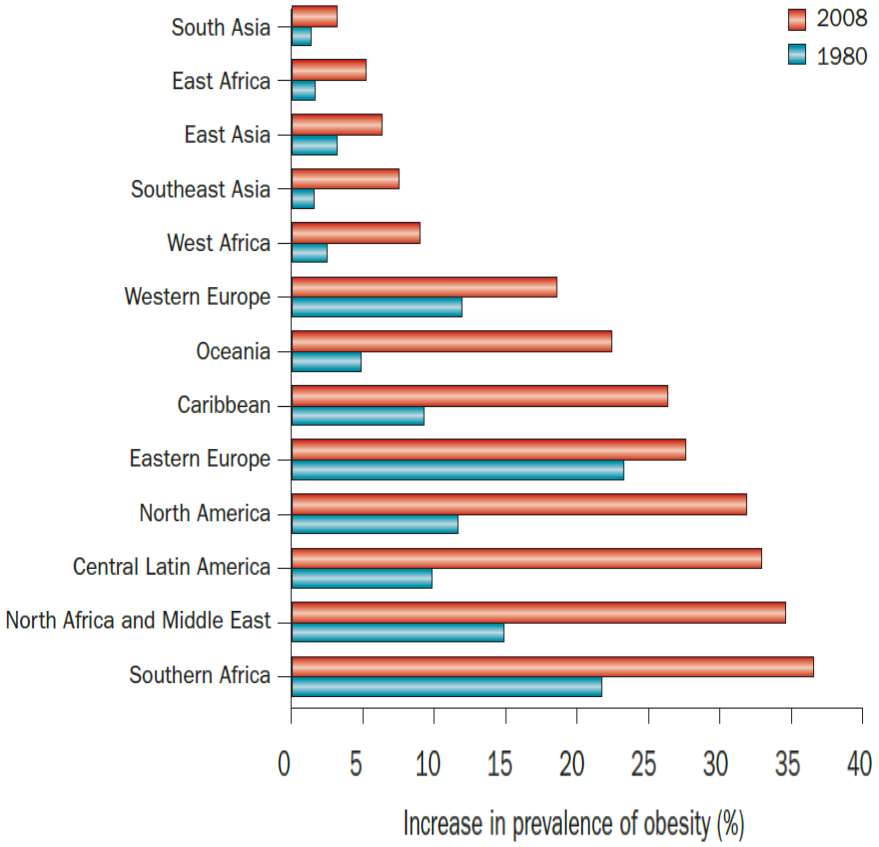
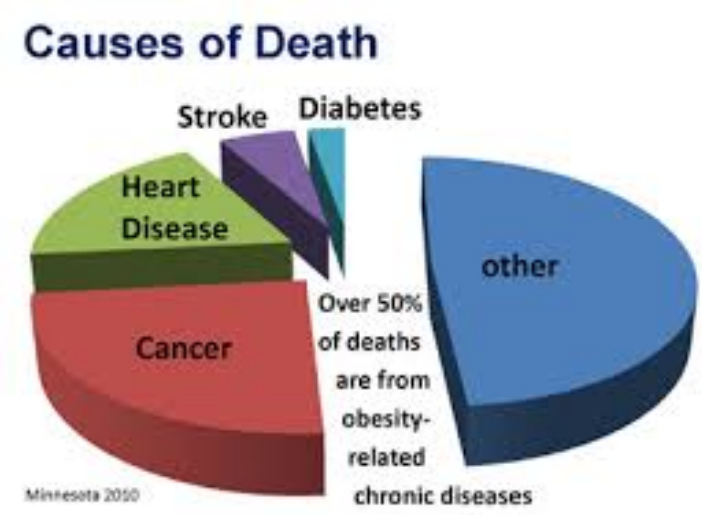
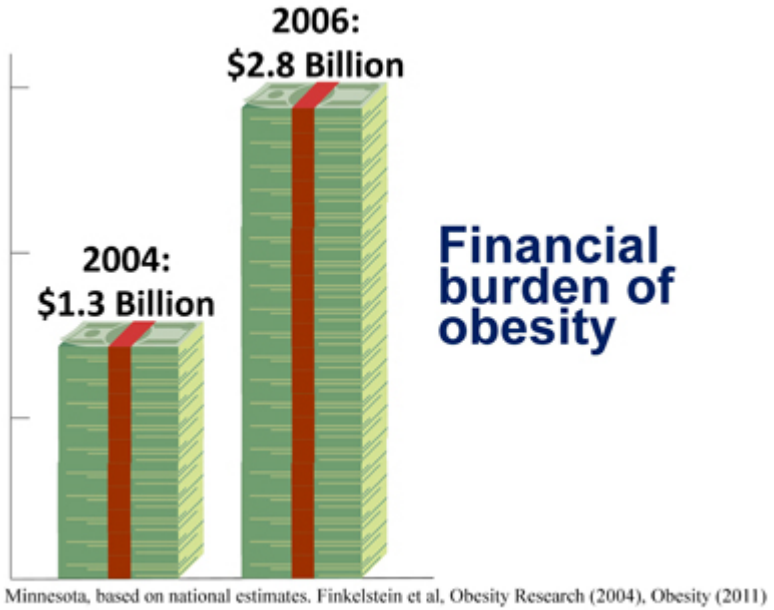
Nutrient excess, obesity and disease burden

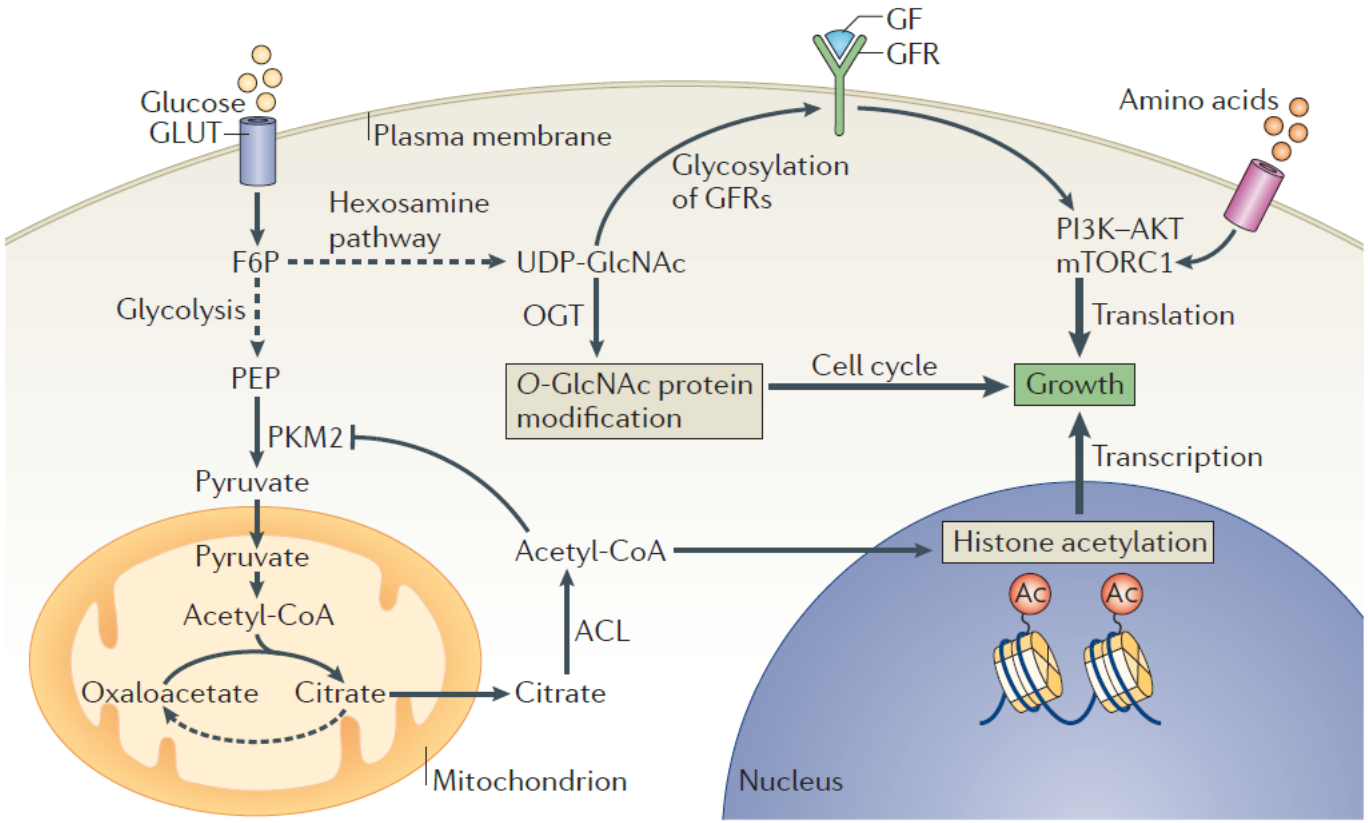


Malik et al, Nat. Rev. Endocrinology 2013





Nutrient overload orchestrate growth programing, in part, via protein acetylation and glycosylation



Wellen and Thompson, Nature Reviews Mol. Cell. Biol. 2012

Lysine Acetylation: an emerging post-translational modification

Timeline

1968-acetylation of histones discovered

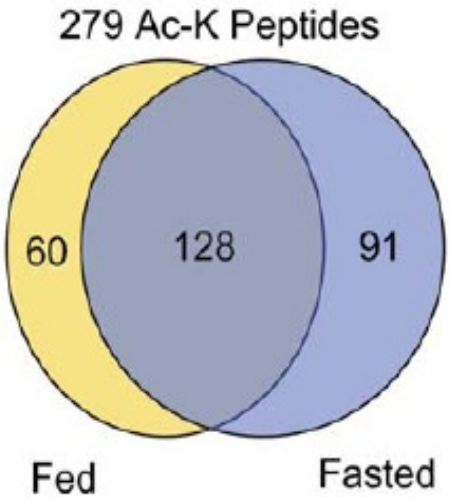
1997: 1st non-histone acetylated protein-p53

Before 2006: <90 proteins are known to be acetylated

2006: 195 acetylated proteins (Kim et al. *Mol. Cell*)

2009: 1750 acetylated proteins (Choudhary et al. *Science*)

2013: > 2000 acetylated proteins

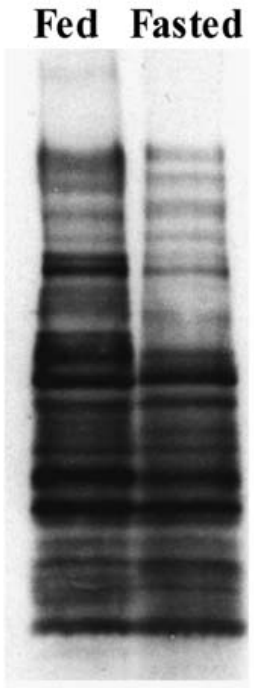


133 mitochondrial proteins (195 total)
20% of mitochondrial proteins

Nutrient availability dependent mitochondrial protein acetylation

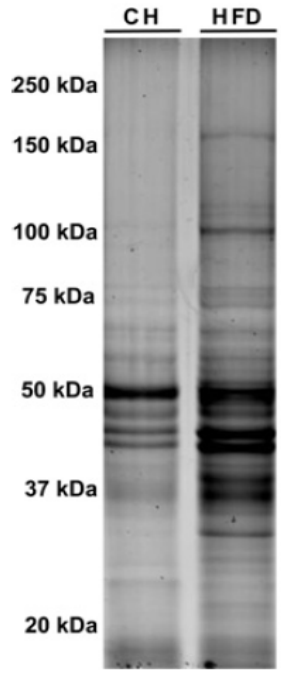
Hepatic Mitochondrial Proteins

Fed/Fasting Comparison



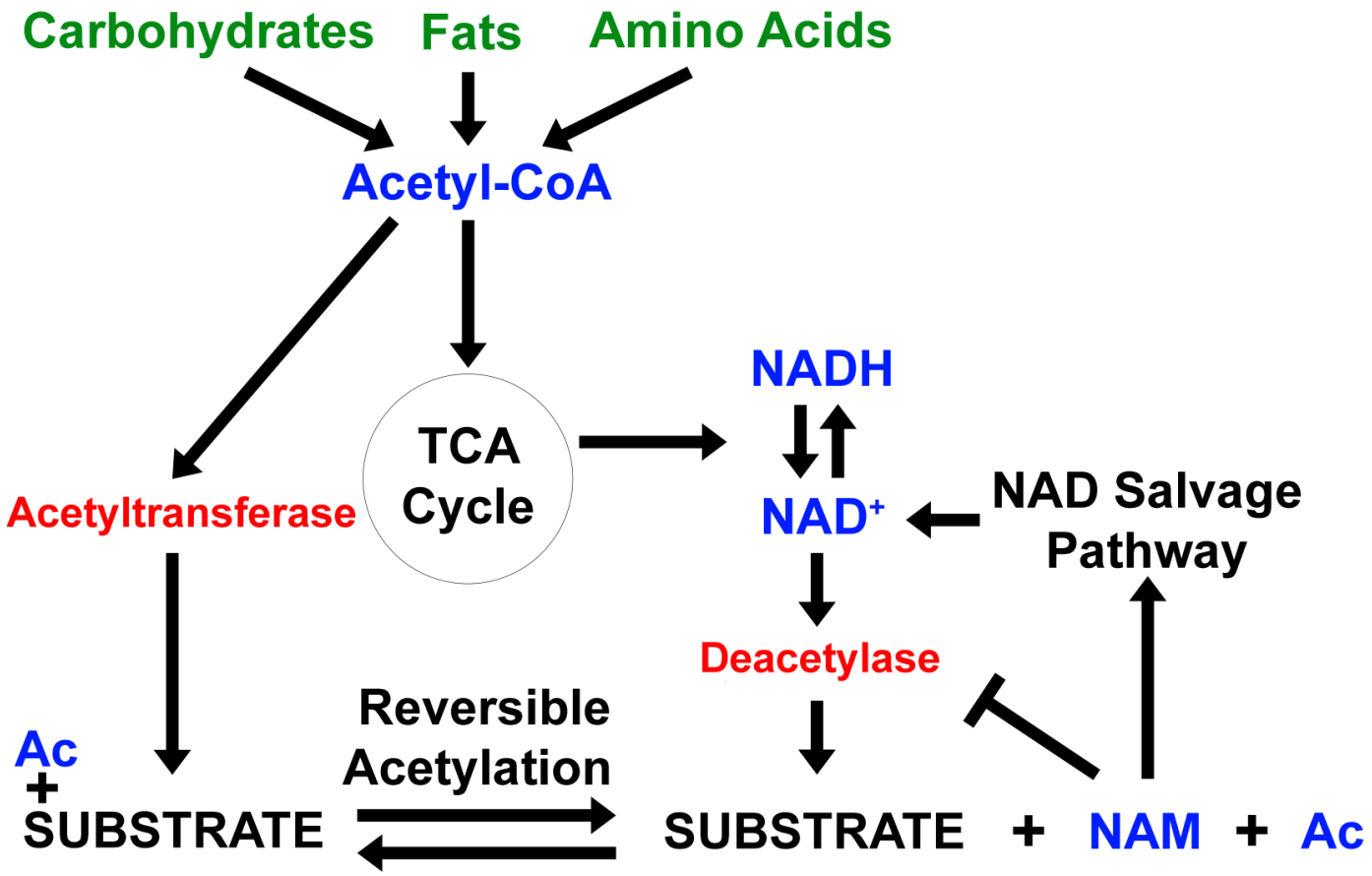
Kim et al, Molecular Cell, 2006

High Fat Feeding



Kendrick et al, Biochem. J. 2011

Acetylation and deacetylation are enzymatically regulated



Scott (2012) *Essays Biochem.*

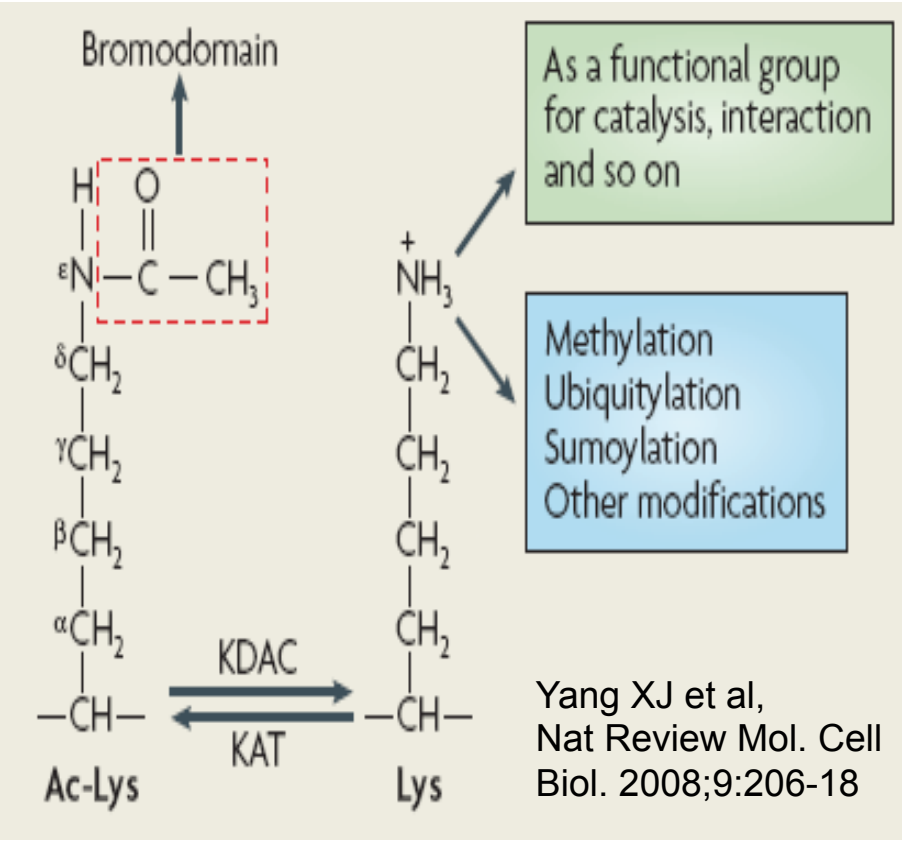
Sirtuins: NAD$^+$-dependent deacetylases

Mammals express seven Sirtuins (Sirt 1-7)

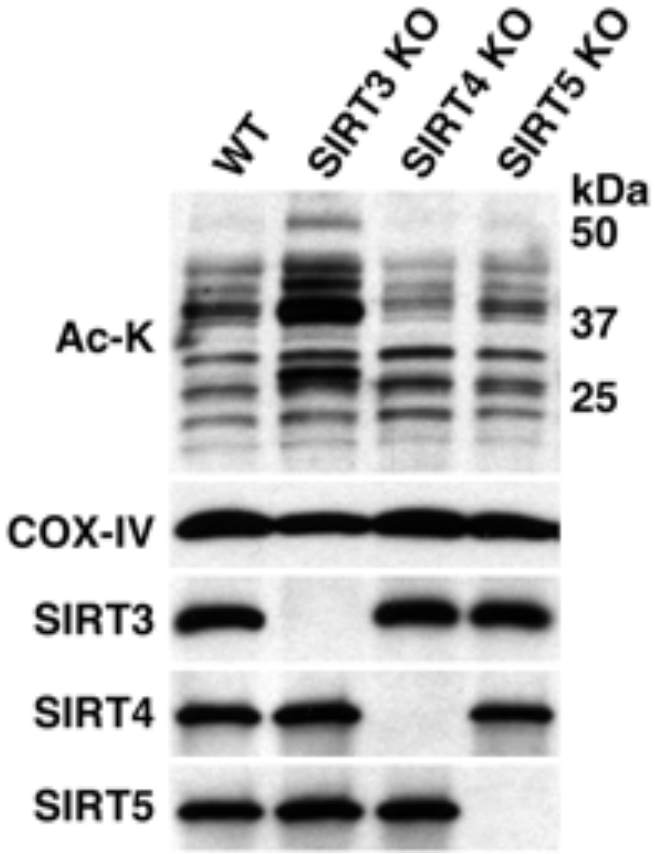
Various cellular localizations:

Sirt1 & 2: nuclear and cytosolic
Sirt3-5: mitochondrial
Sirt6 & 7: nuclear

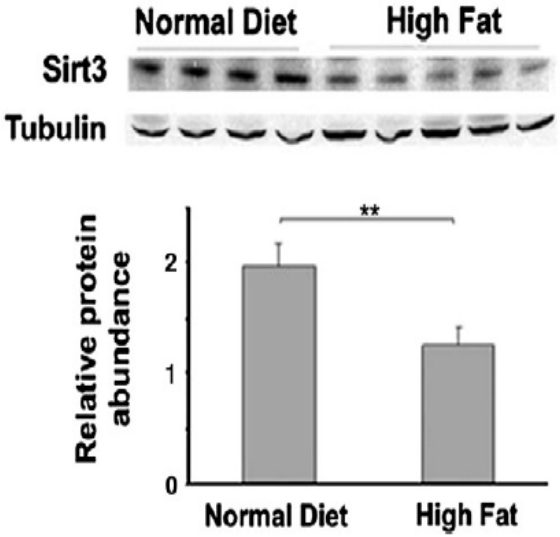
NAD$^+$-dependent deacetylation
ADP Ribosylation activity (Sirt4 & 6)



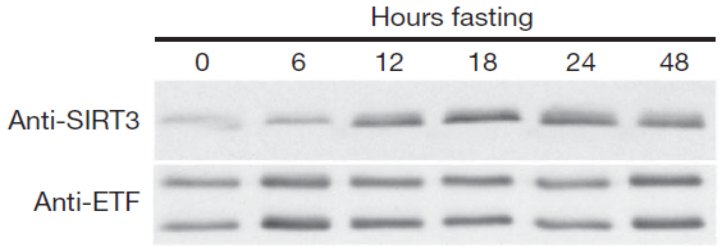
Sirt3 functions as the nutrient-sensitive mitochondrial lysine deacetylase



Lombard et al. (2007) *Mol. Biol. Cell*



Bao J et al. *Free Radical Biol. and Med. (2010)*



Hirschey et al, *Nature* (2010)

Association of Acetaminophen Hepatotoxicity With Fasting and Ethanol Use

David C. Whitcomb, MD, PhD, Geoffrey D. Block, MD, MPH

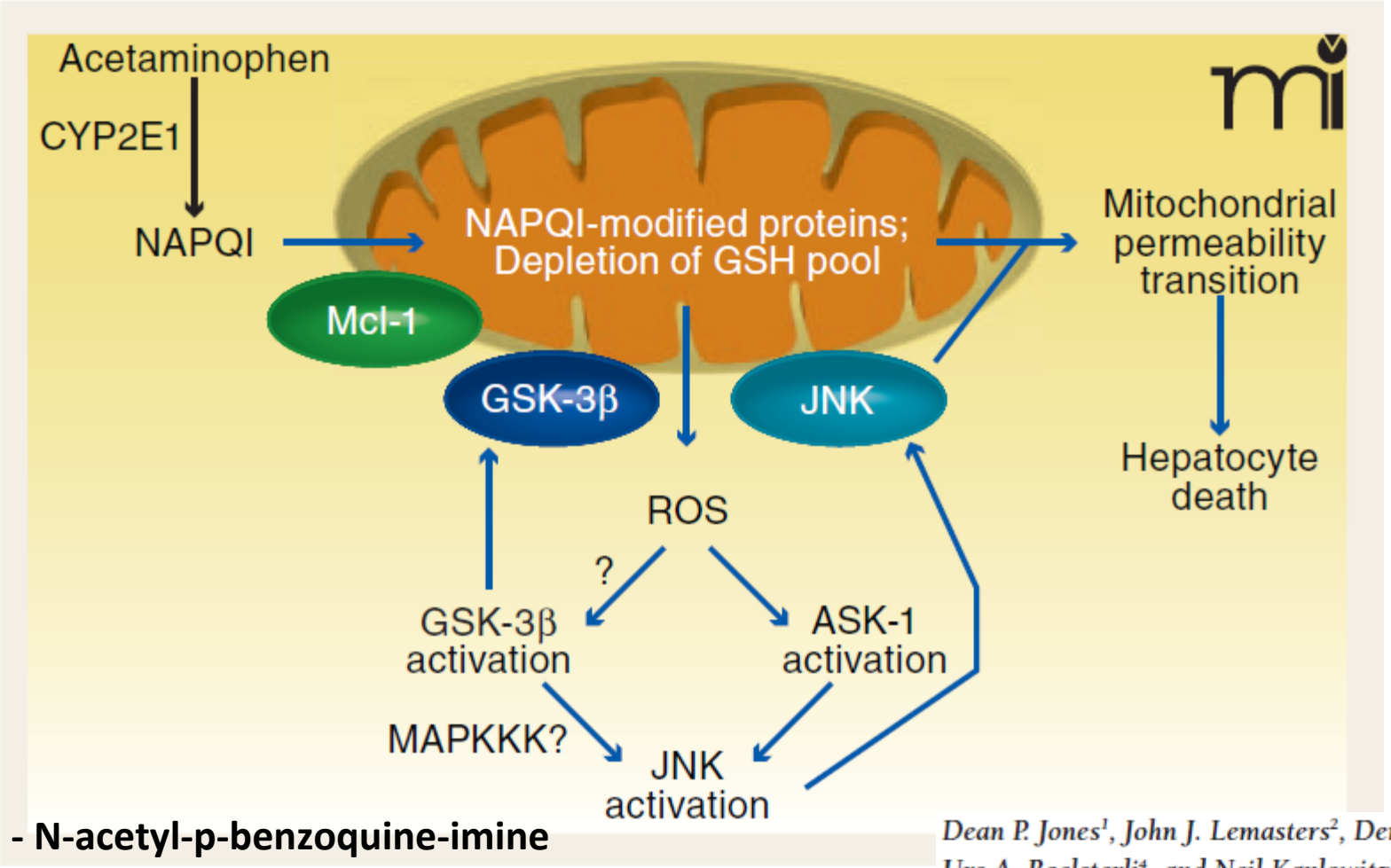
JAMA, December 21, 1994—Vol 272, No. 23

Recent fasting was more common than recent alcohol use among those who suffered hepatotoxicity after a dose of 4 to 10 g of acetaminophen per day (P=.02). Recent alcohol use was more common in the group who took more than 10 g/d than in those who took 4 to 10 g/d (P=.004).

Conclusion: Acetaminophen hepatotoxicity after a dose of 4 to 10 g/d was associated with fasting and less commonly with alcohol use. Patients who developed hepatoxicity after taking acetaminophen doses of greater than 10 g/d for therapeutic purposes were alcohol users. Acetaminophen hepatotoxicity after an overdose appears to be enhanced by fasting in addition to alcohol ingestion.

Acetaminophen toxic metabolites can bind to lysine residues

Whether this plays a role in hepatotoxicity is unknown



NAPQI - N-acetyl-p-benzoquine-imine

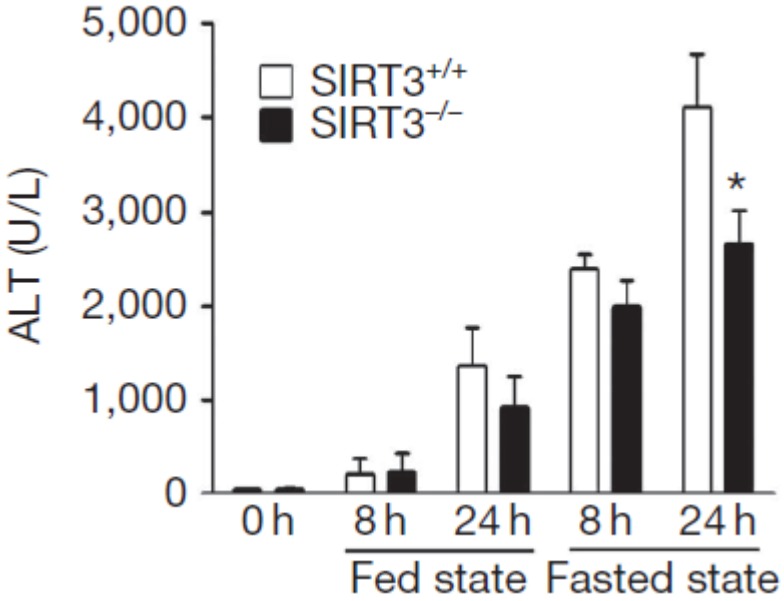
Dean P. Jones[1], John J. Lemasters[2], Derick Han[3], Urs A. Boelsterli[4], and Neil Kaplowitz[3]

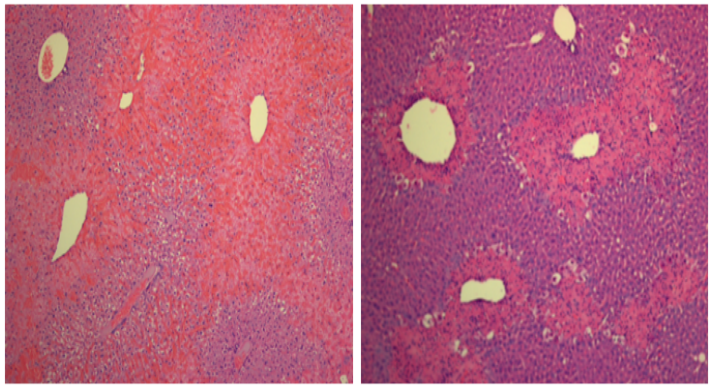
Hypothesis

The level of mitochondrial protein acetylation modulates susceptibility to acetaminophen liver injury

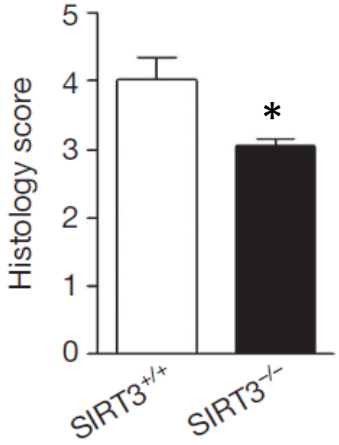
This may be mediated in part by modulating NAPQI binding to mitochondrial proteins?

SIRT3 KO mice are resistant to acetaminophen-induced liver injury





SIRT3$^{+/+}$ SIRT3$^{-/-}$

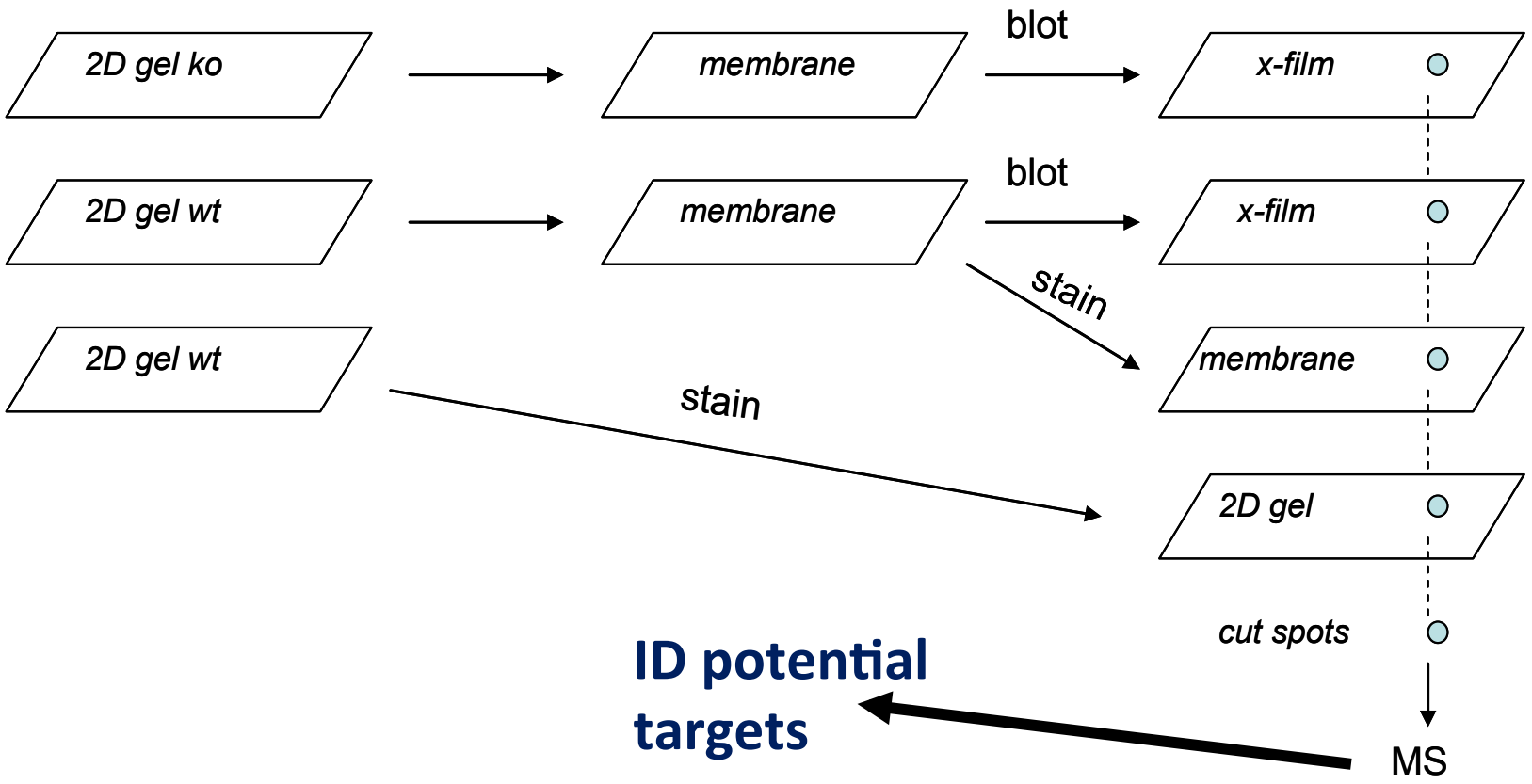


APAP 350mg/kg IP administration

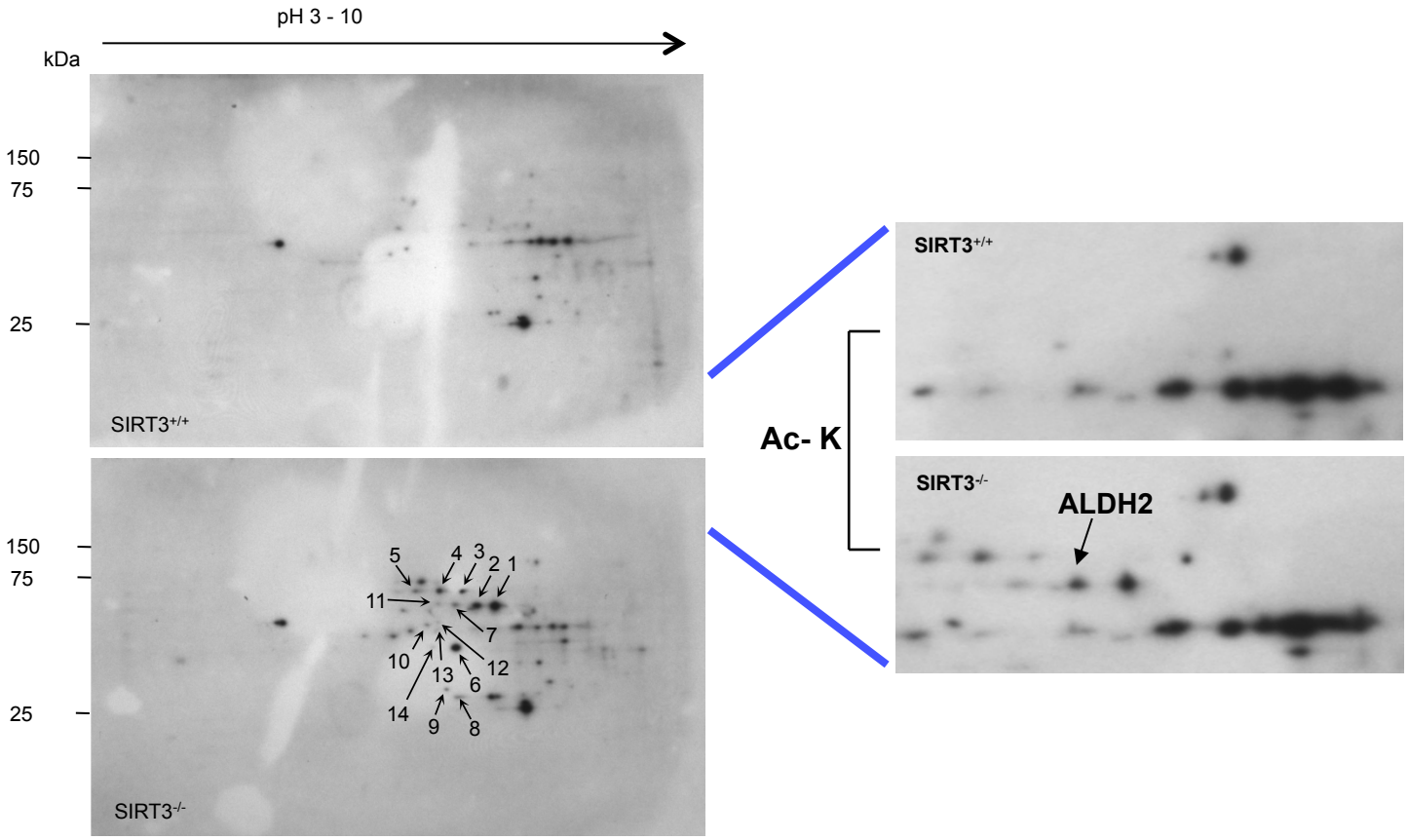
N-acetyl-p-aminophenol

Identification and characterization of novel substrates of SIRT3 in the murine liver

2D gel and MS to ID hyper-acetylated mitochondrial proteins



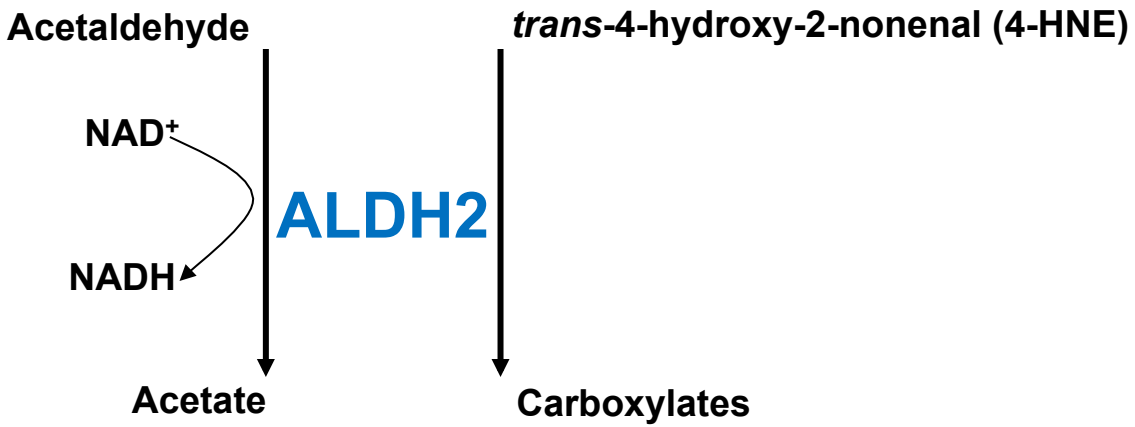
Representative 2-D gels employing an antibody directed against acetylated lysine-residues



Major ALDH2 metabolic pathways

Ethanol oxidation

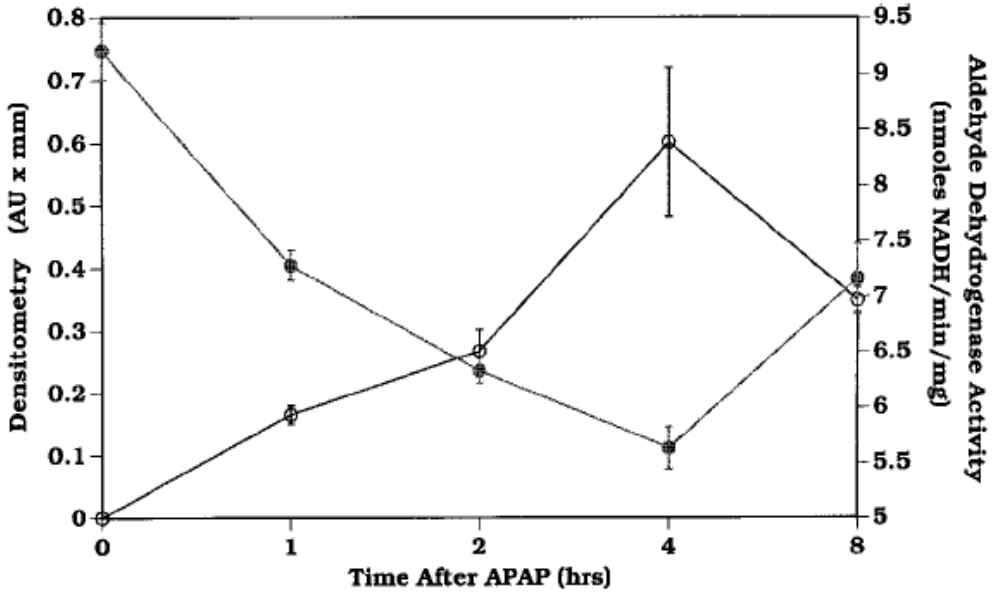
Lipid peroxidation produces α,β-unsaturated hydroxyalkenal



Catalyze the oxidation of aldehydes

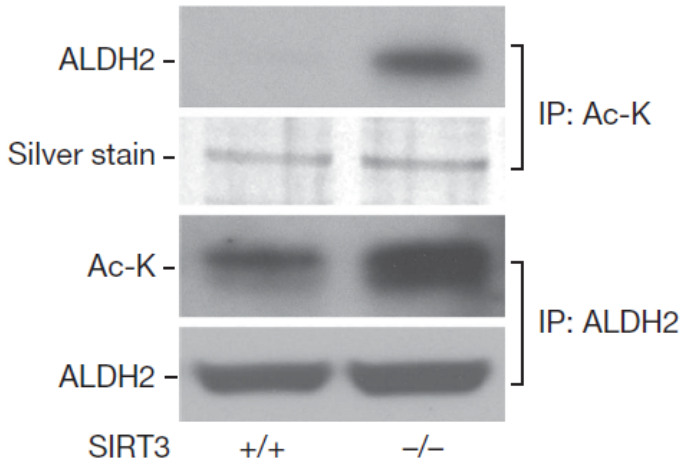
Mitochondrial ALDH2 is a direct target of the toxic acetaminophen metabolite - NAPQI

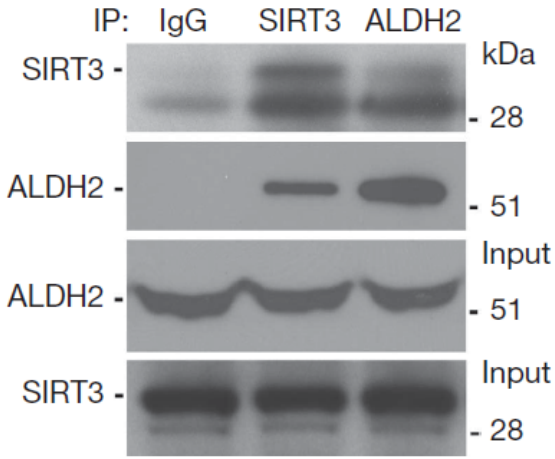
Is this interaction integral to APAP hepatotoxicity?

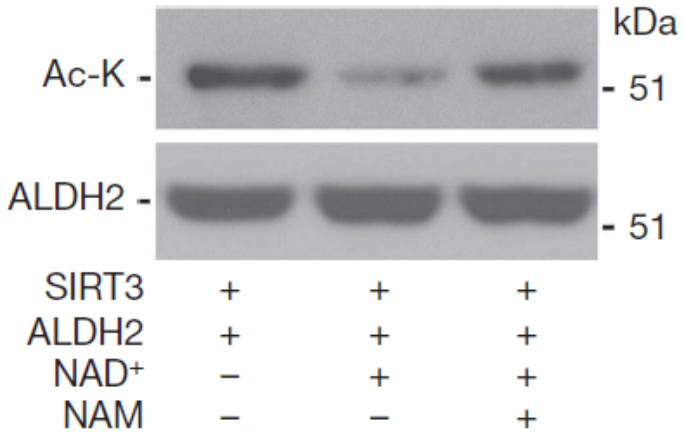


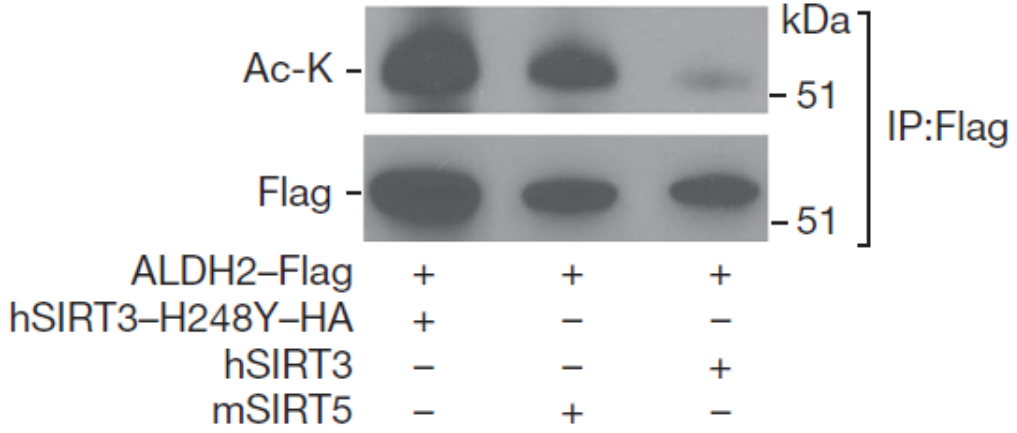
Landin JS, et al. Toxicology and Applied Pharmacology 1996;141:299-307

ALDH2 is a substrate for Sirt3 Deacetylation

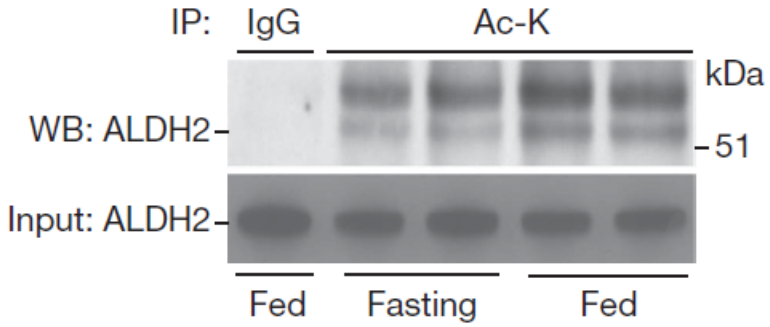


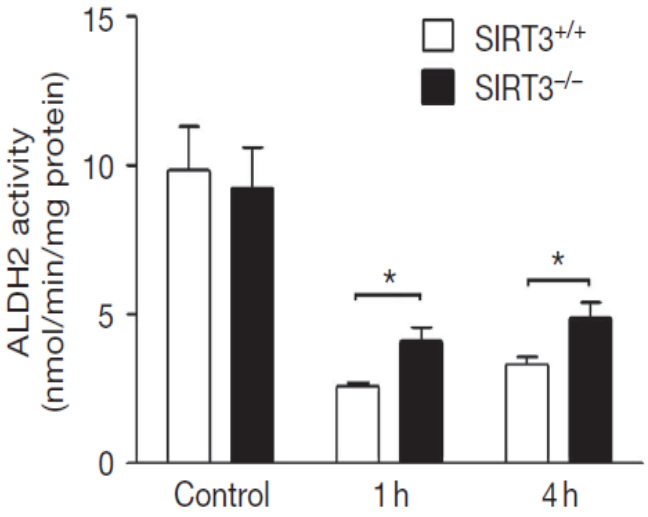


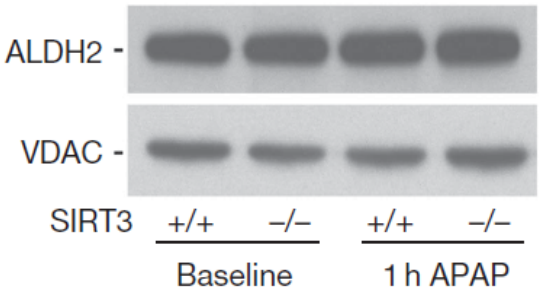


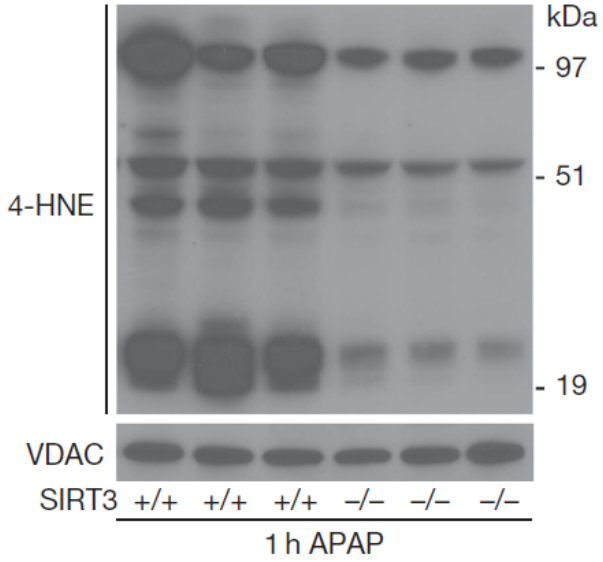


ALDH2 function is preserved in fasting Sirt3 KO mice

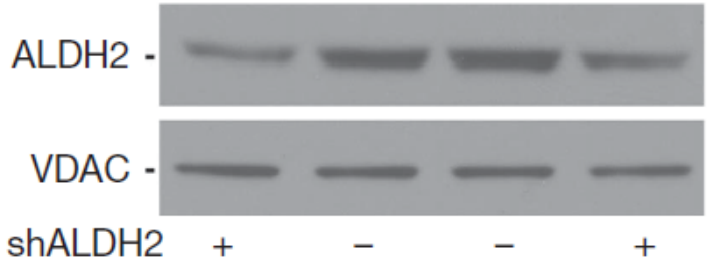


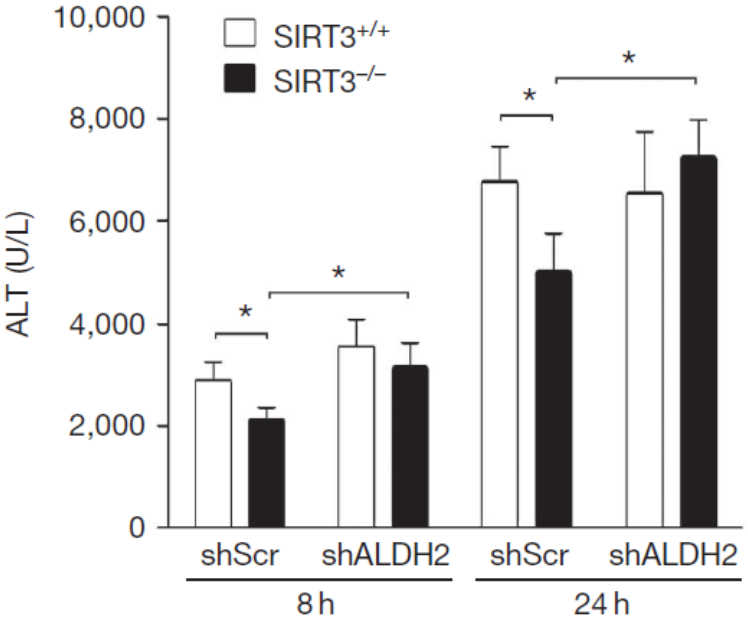


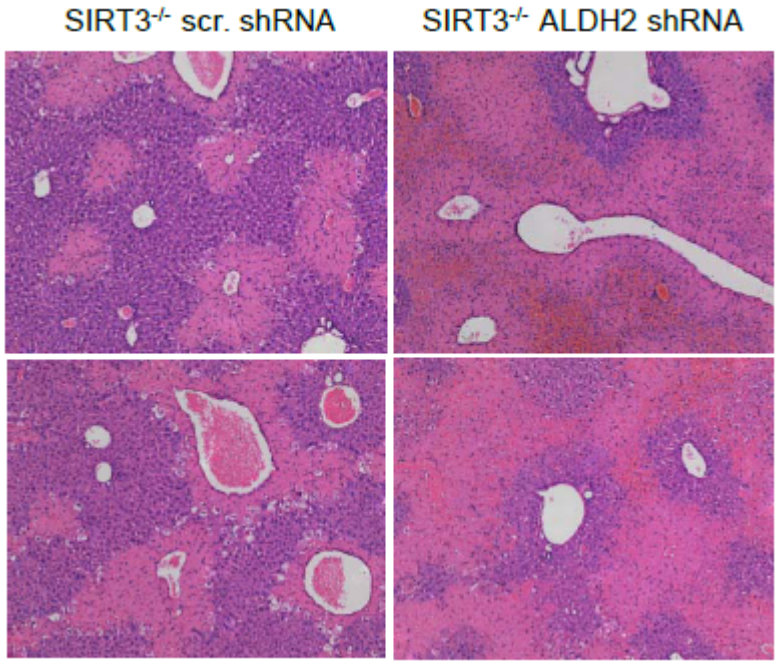




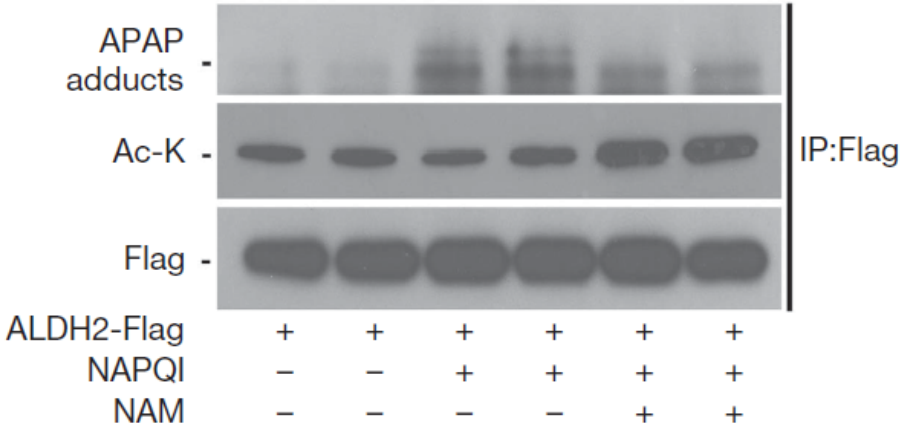
Hepatic shRNA knockdown of ALDH2 negates acetaminophen 'resilience' in SIRT3 -/- mice

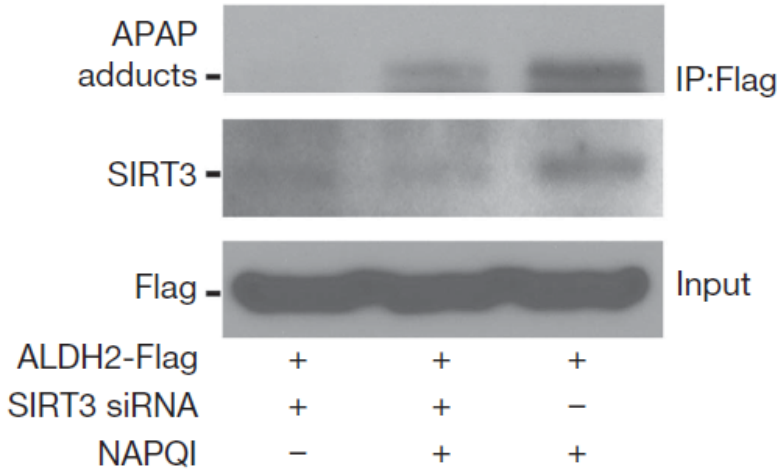


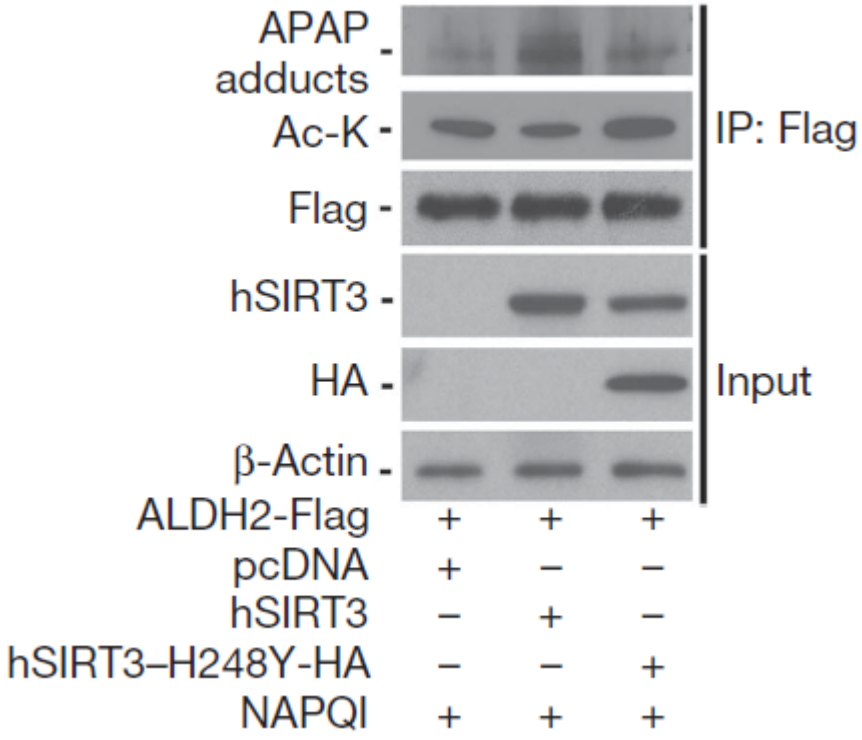




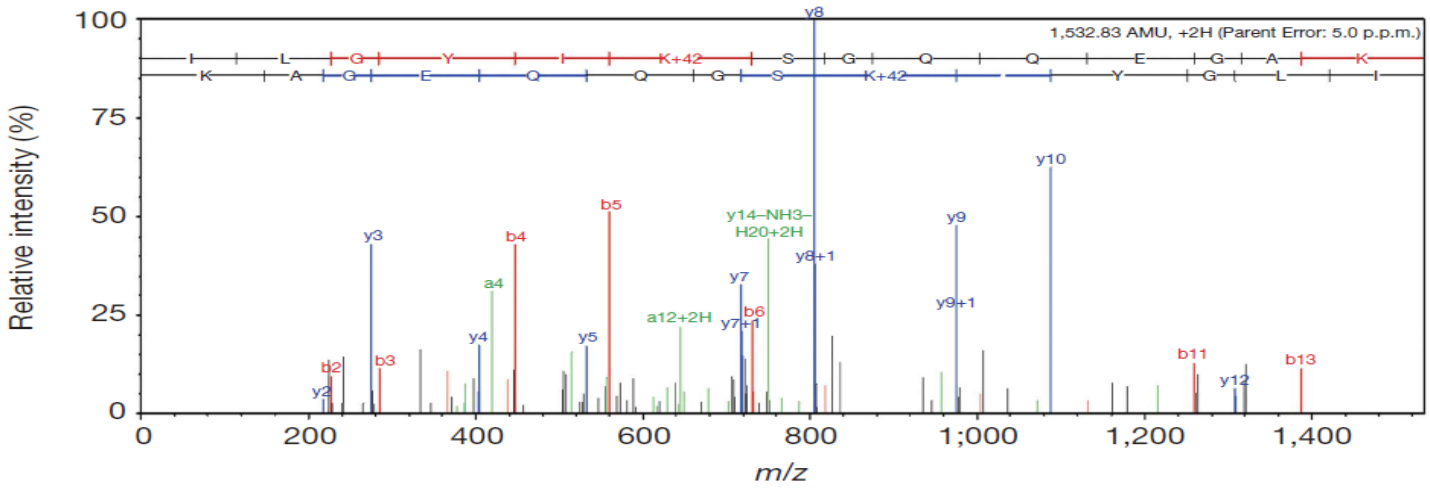
Sirt3-dependent acetylation status modulates NAPQI binding to ALDH2

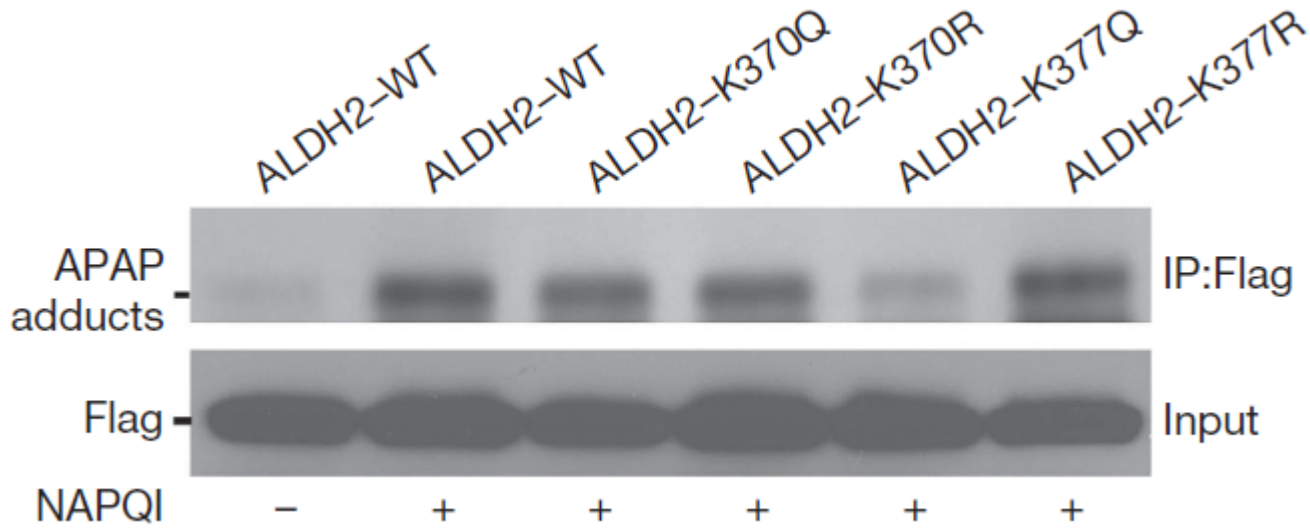




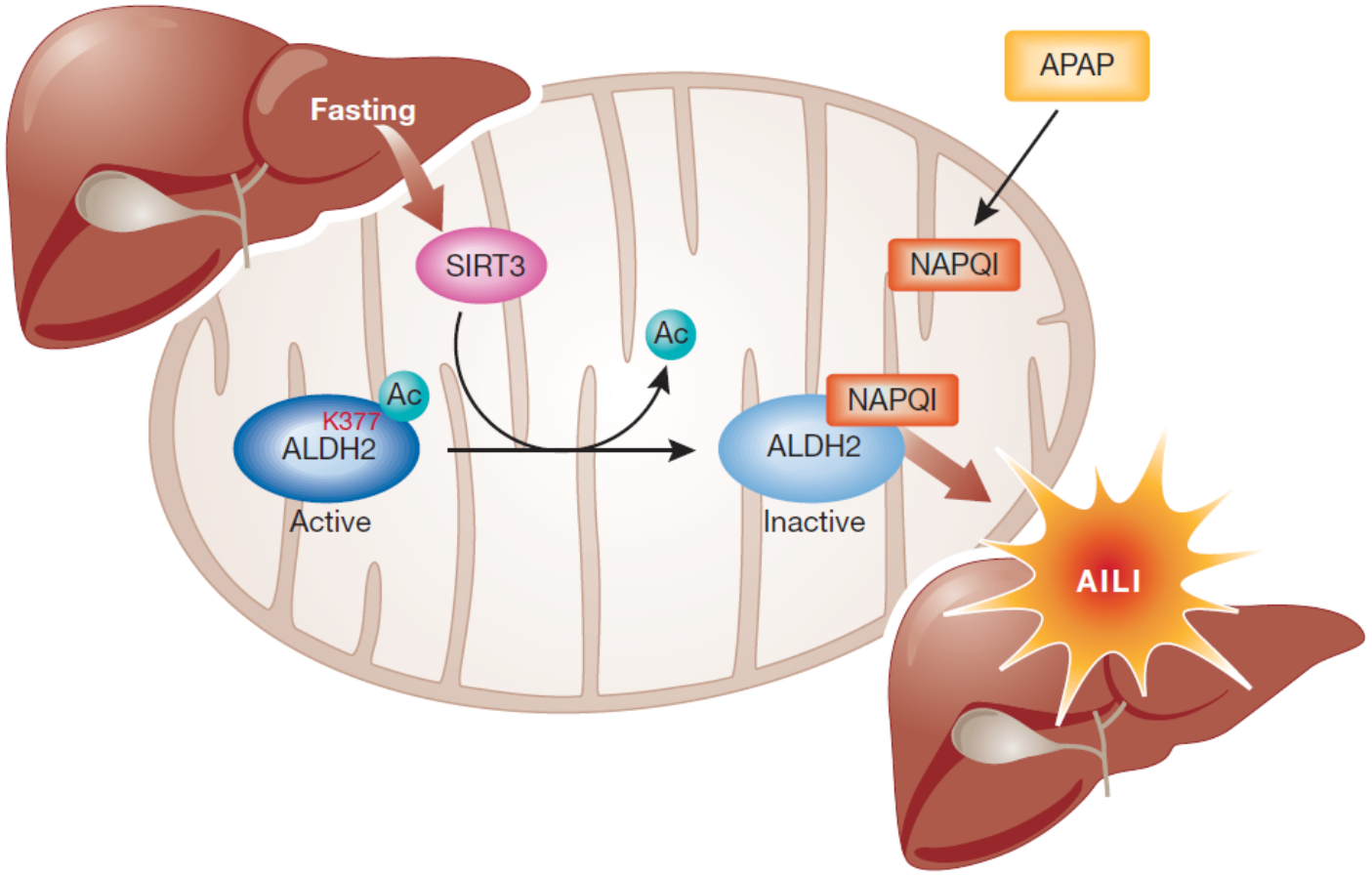


Identification of ALDH2- K377 as the functional residue for NAPQI binding

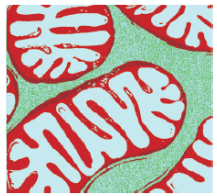




Identification of an allosteric role of lysine deacetylation in fasting susceptibility to acetaminophen injury



Lu et al. EMBO Reports 2011



Talk Outline

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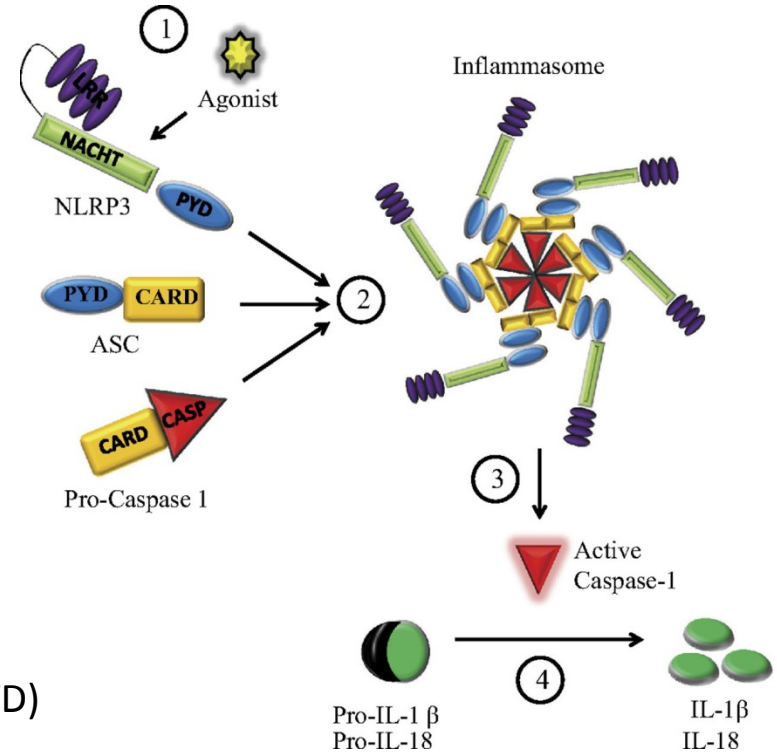
- Fasting and Tylenol Liver Toxicity

- Sirtuin Biology and Mitochondrial Quality Control
- The Role of Fasting and Sirt3 on NLRP3 Inflammasome Biology

NIH National Heart, Lung, and Blood Institute

Defining the Inflammasome Program

Inflammasome: Multiprotein intracellular complex that sense pathogen / damage associated molecular patterns (PAMPS/DAMPs) and activate caspase-1, which in turn cleaves/activates pro-inflammatory cytokines IL-1β and IL-18.



NLRP - Nod-like receptor family protein
ASC - Adaptor apoptosis-associated speck-like protein containing a CARD and pyrin domain (PYD)

Sutterwala et al. (2014) Ann N Y Acad Sci

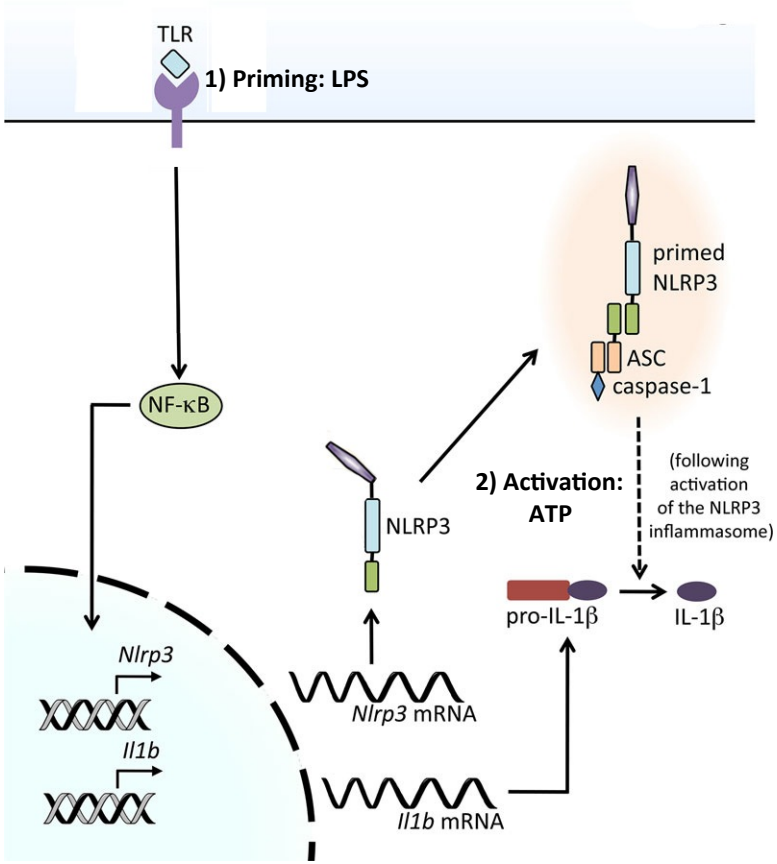
The NLRP3 Inflammasome Program

NLRP3 Inflammasome:

- Multiple triggers (**'sterile inflammation'**) – asthma, atherosclerosis, DM and aging
- Regulated at the transcript and post-translational levels:

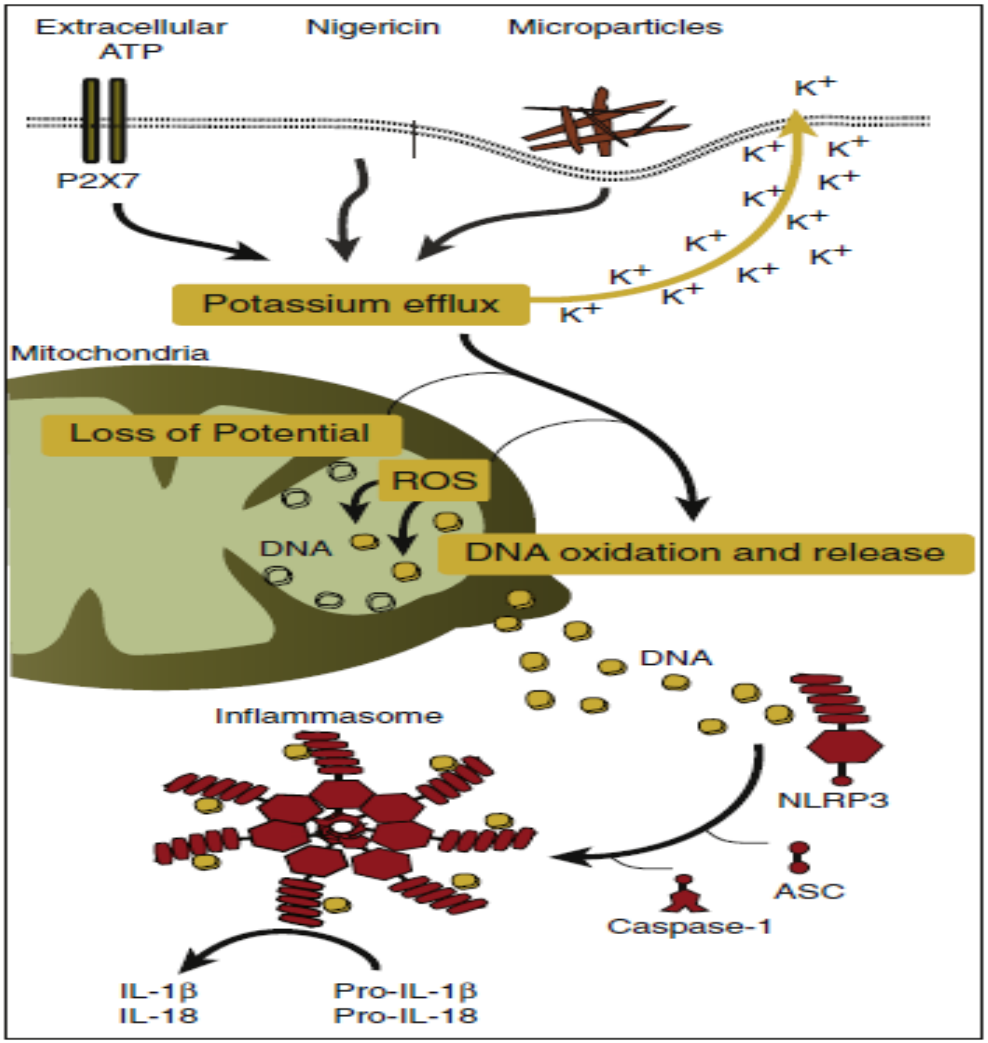
Priming: Transcriptional induction of genes encoding components of the NLRP3 complex.

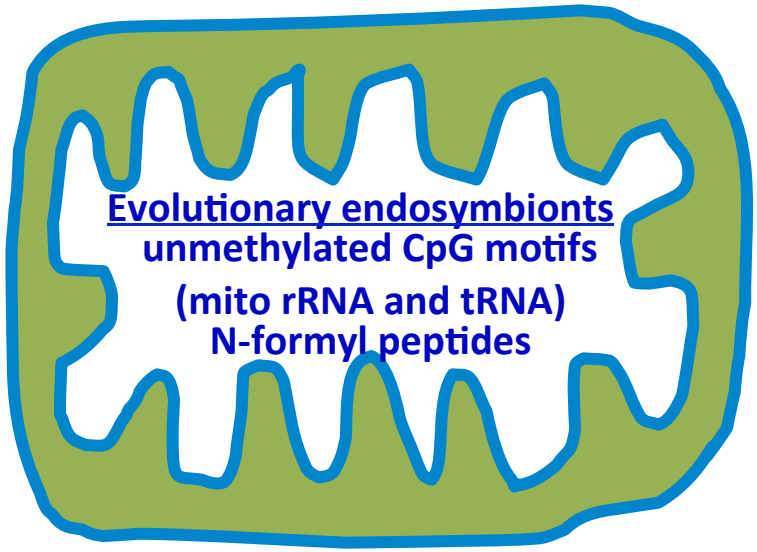
Activation: Complex activation by stress-signals - ATP, nigericin, fatty acids & cholesterol crystals.



Sutterwala et al. (2014) Ann N Y Acad Sci

Mitochondrial Disruption as a Disease Associated Molecular Pattern (DAMP) in NLRP3 Activation





Martinon, Immunity 2012

Available online at www.sciencedirect.com
ScienceDirect
Free Radical Biology & Medicine 42 (2007) 665–674
www.elsevier.com/locate/freeradbiomed

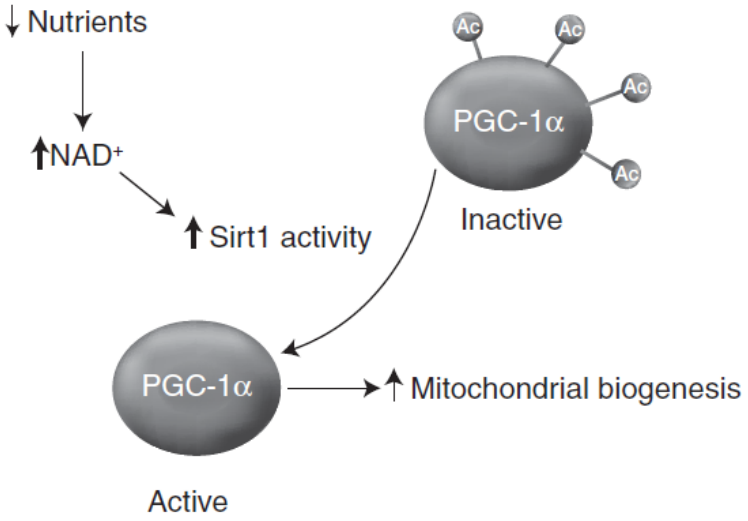
Original Contribution

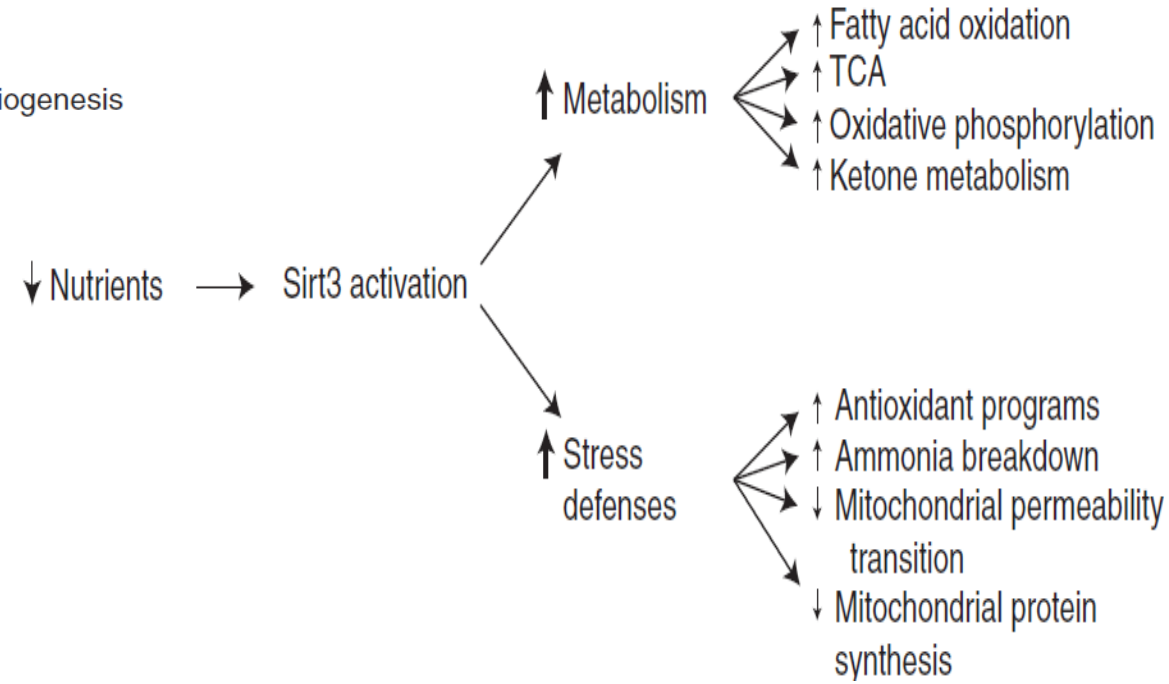
Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma

James B. Johnson [a,*], Warren Summer [b], Roy G. Cutler [c], Bronwen Martin [c], Dong-Hoon Hyun [c], Vishwa D. Dixit [d], Michelle Pearson [c], Matthew Nassar [c], Richard Tellejohan [c], Stuart Maudsley [c], Olga Carlson [e], Sujit John [f], Donald R. Laub [g], Mark P. Mattson [c]

Sirt1 and Sirt3 Deacetylase Enzymes

Modulate Mitochondrial Function/Quality
(Caloric Restriction/Fasting)



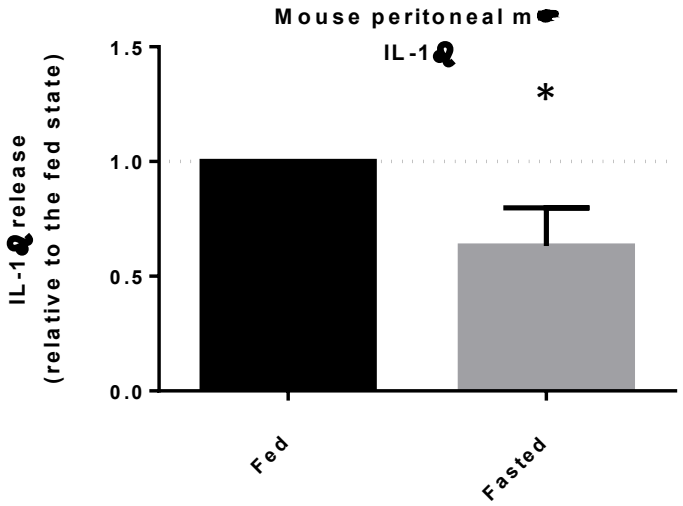


Sack MN and Finkel T.
Cold Spring Harb Perspect Biol
2012

Can Mitochondrial Sirtuins Regulate the NLRP3 Inflammasome?

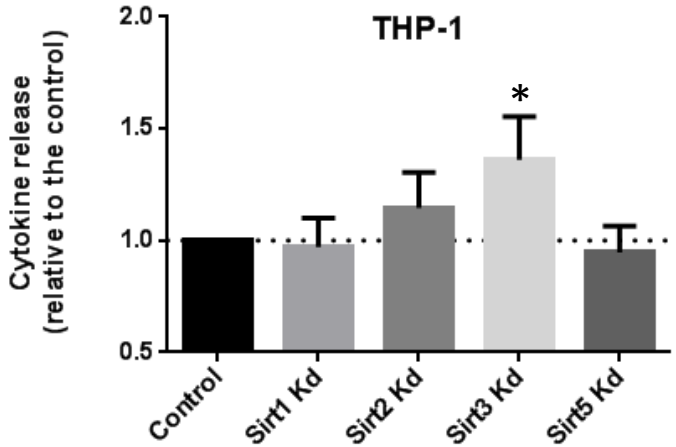
Is this Dependent on the Modulation of Mitochondrial Homeostasis?

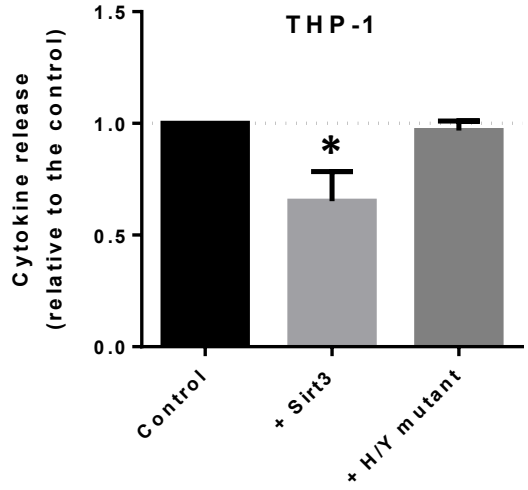
Fasting (caloric restriction mimetic) suppresses the NLRP3 inflammasome?



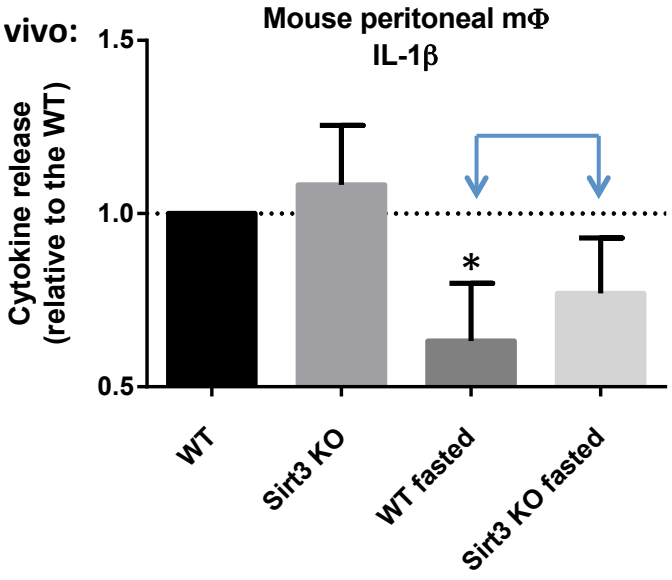
There is a 40 % decrease in the release of IL-1β after fasting

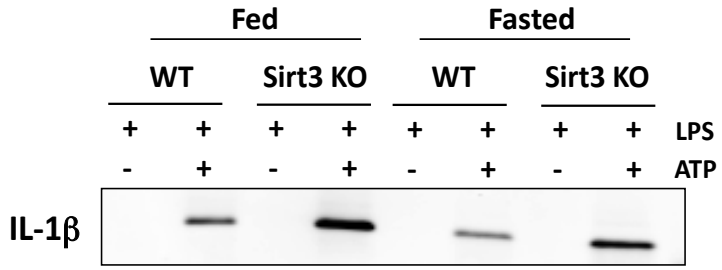
NLRP3 activation is modulated by Sirt3



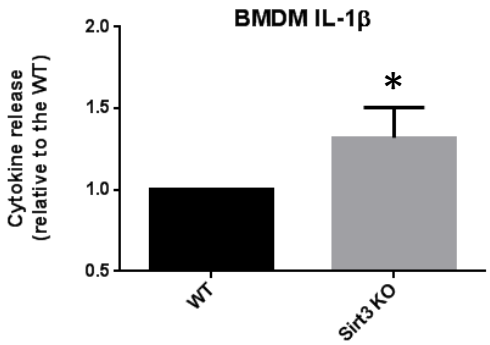


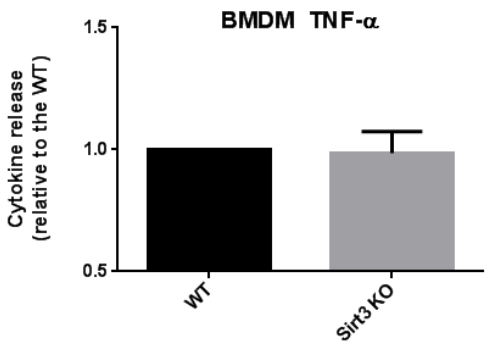
And in mouse in vivo:



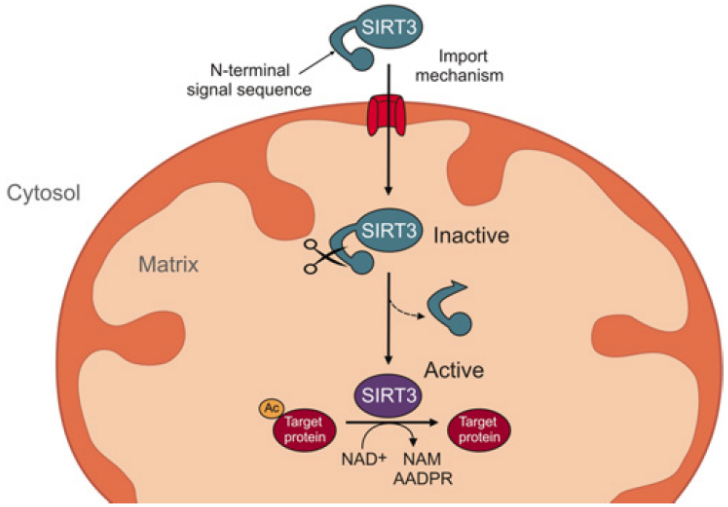


The role of Sirt3 in NLRP3 inflammasome regulation is confirmed in Bone Marrow Derived Macrophages



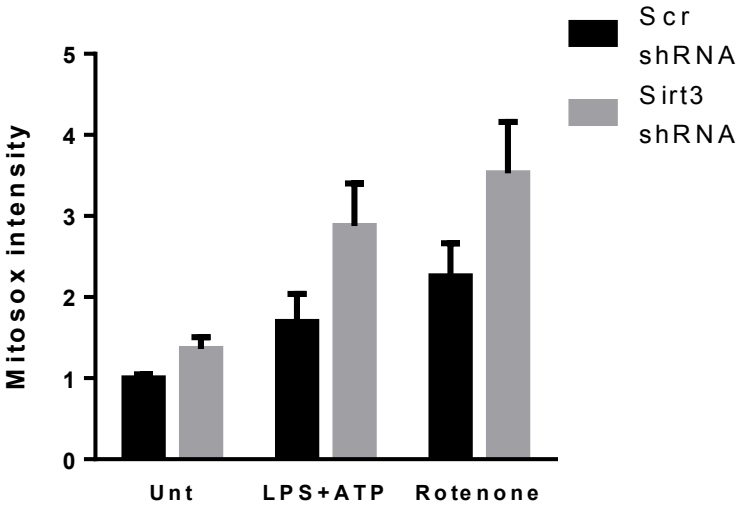


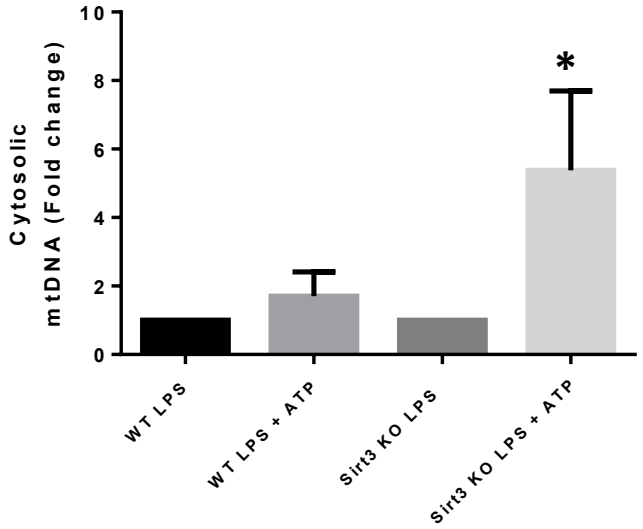
Mitochondrial Homeostatic Role of Sirt3



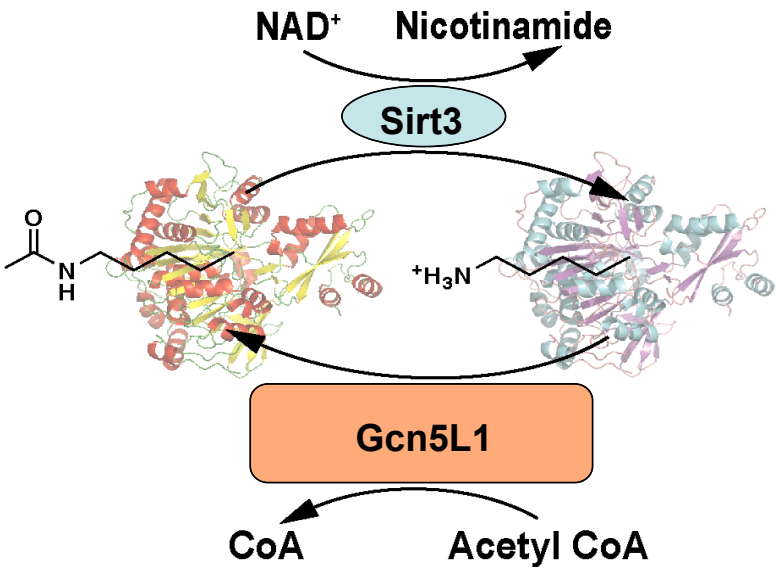
Giralt A et al. Biochem J, 2012

Improve Energetics
Decrease ROS
MPT Resistance
Diminished Apoptosis
Enhanced Mitophagy



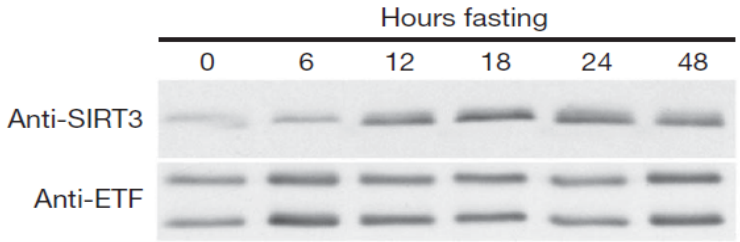


The counter-regulatory control of mitochondrial protein acetylation



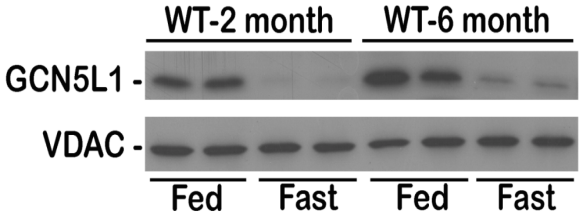
Scott I, et al. Biochem J. 2012

Sirt3 levels are increased with fasting



Hirschey et al, Nature 2010

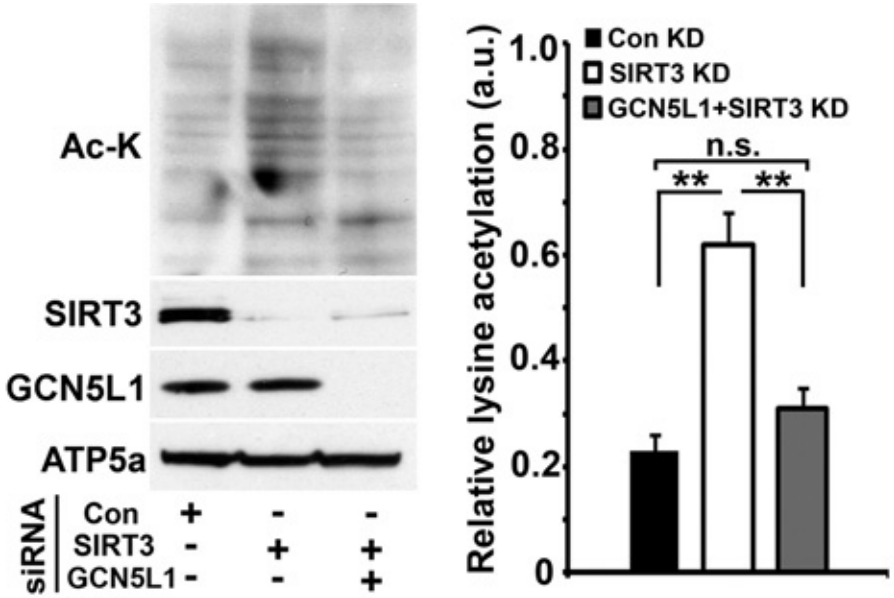
Gcn5L1 levels are decreased with fasting



Webster B et al. J. Cell Science (2013)

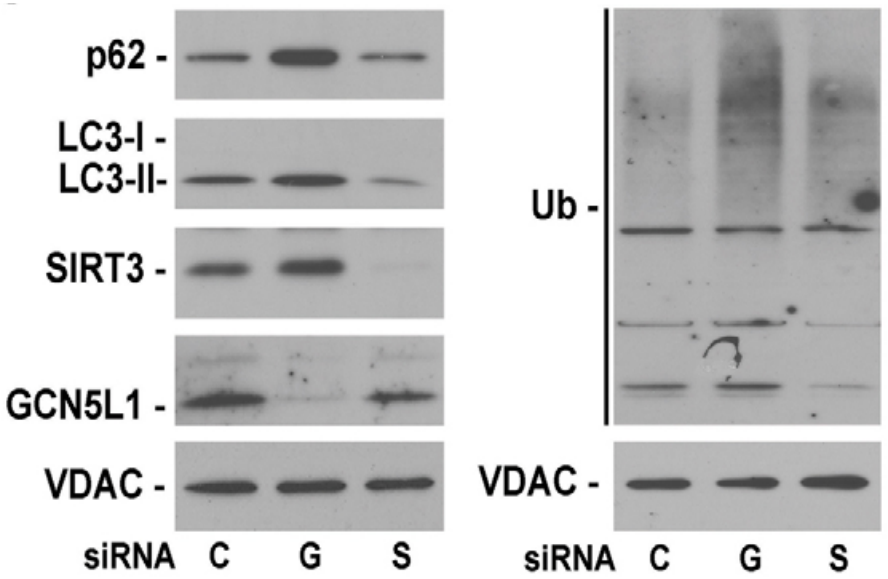
Counter-regulatory roles of Gcn5L1 and Sirt3 on mitochondrial acetylation and function

Mitochondrial Protein Acetylation



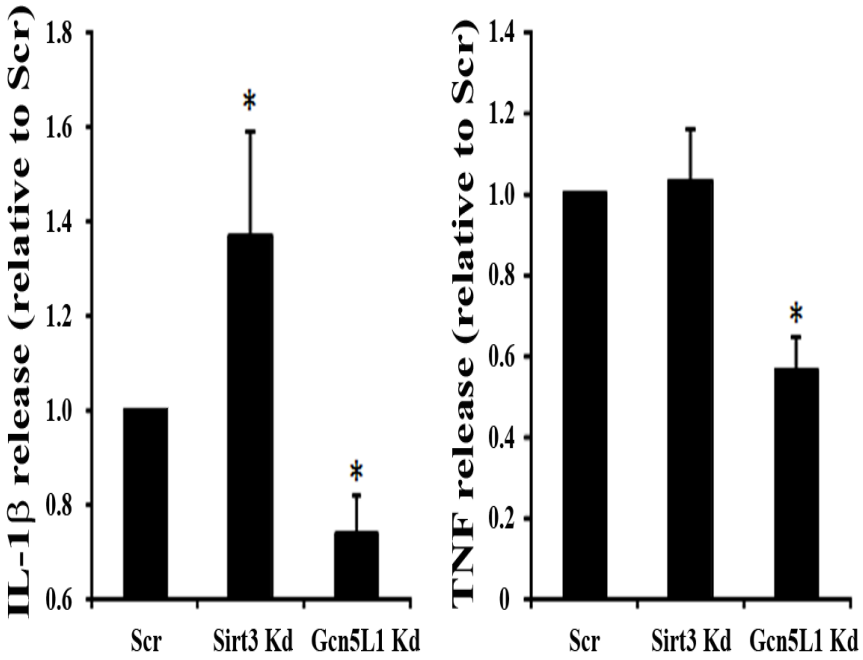
Scott I, et al. Biochem J. 2012

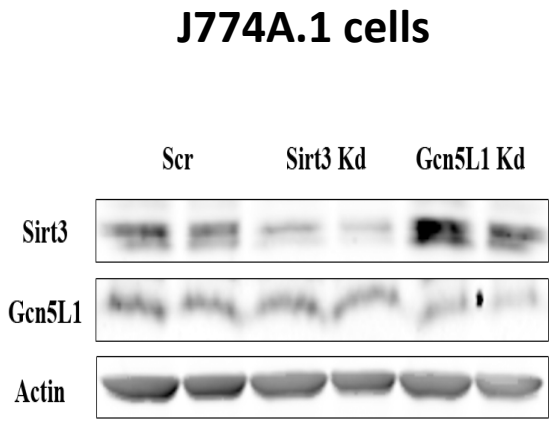
Modulation of Mitophagy



Webster B, Scott I. J Cell Sci 2013

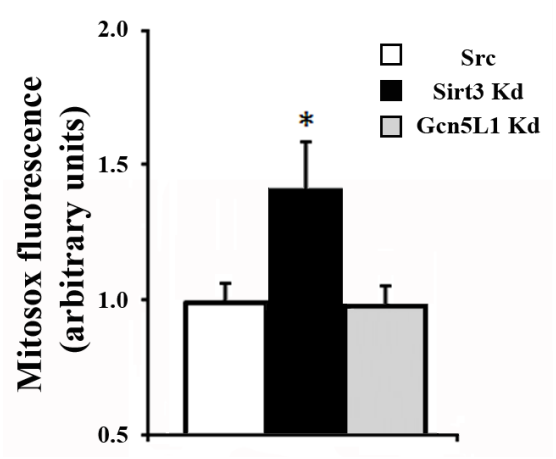
Gcn5L1 and Sirt3 depletion have counter-regulatory effects on the NLRP3 inflammasome

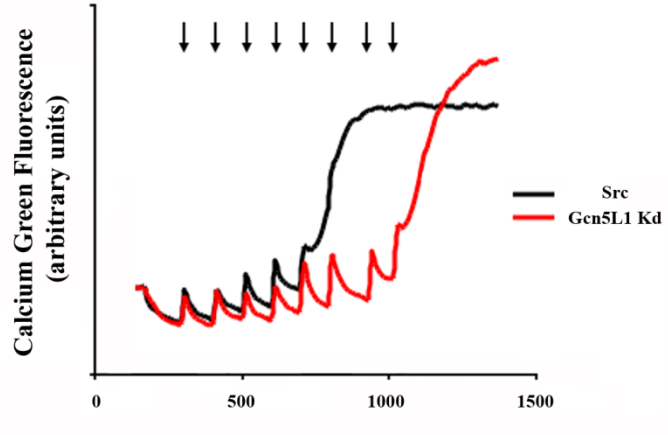


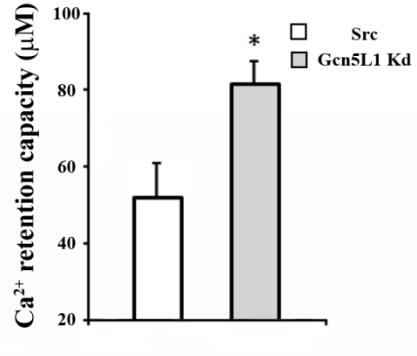


Traba J, et al. *Unpublished Data*

Mitochondrial phenotype in J774A.1 macrophages

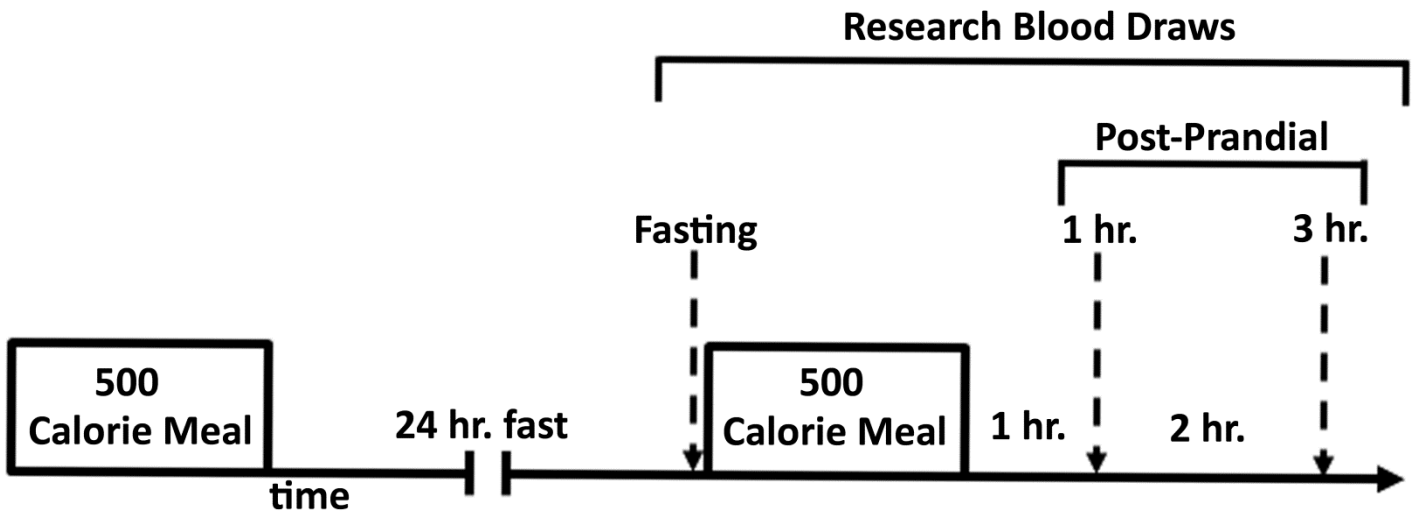




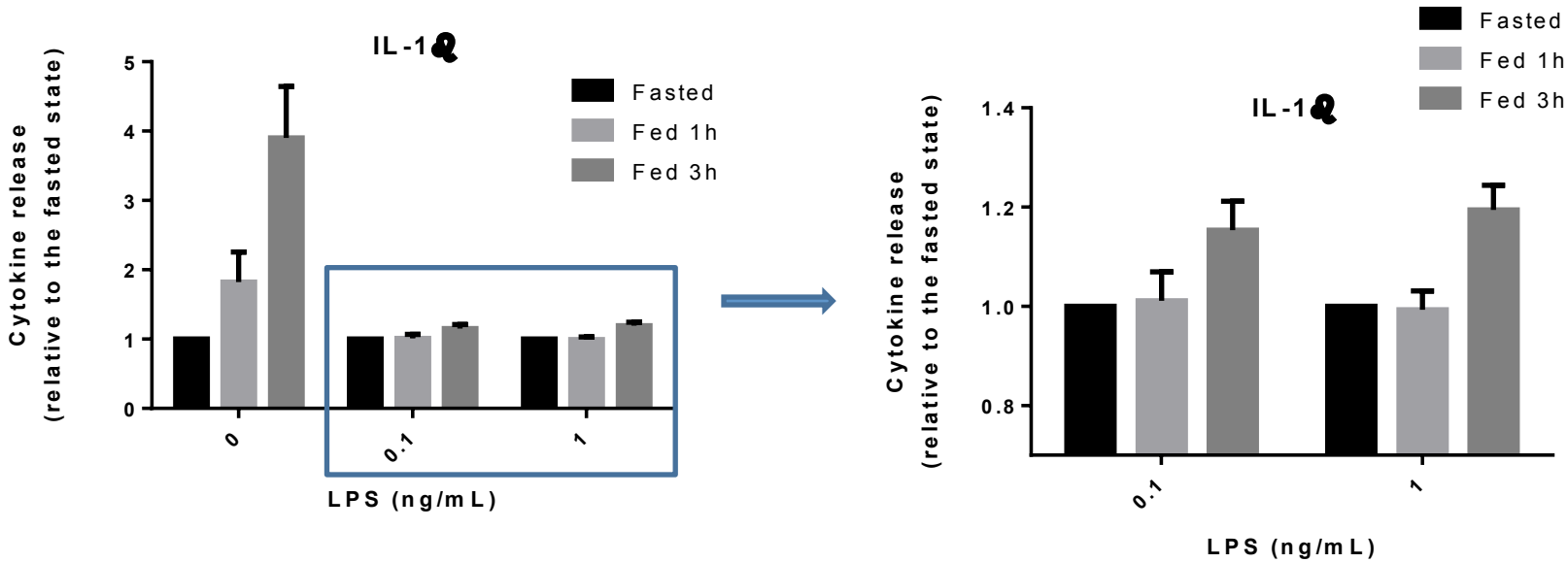


Traba J, et al. *Unpublished Data*

NHLBI Protocol: *Pilot Study to Evaluate the Effect of Fasting on the NLRP3 Inflammasome*



Fasting Blunts the NLRP3 inflammasome in Human Subjects



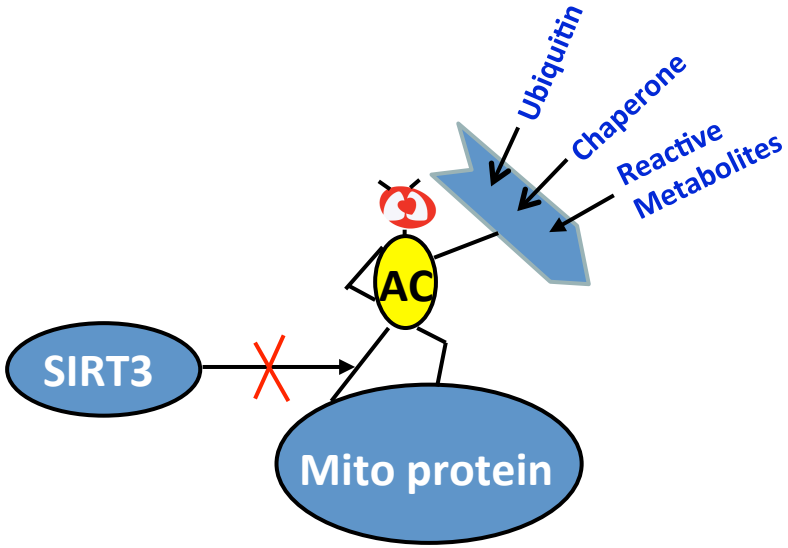
Conclusions

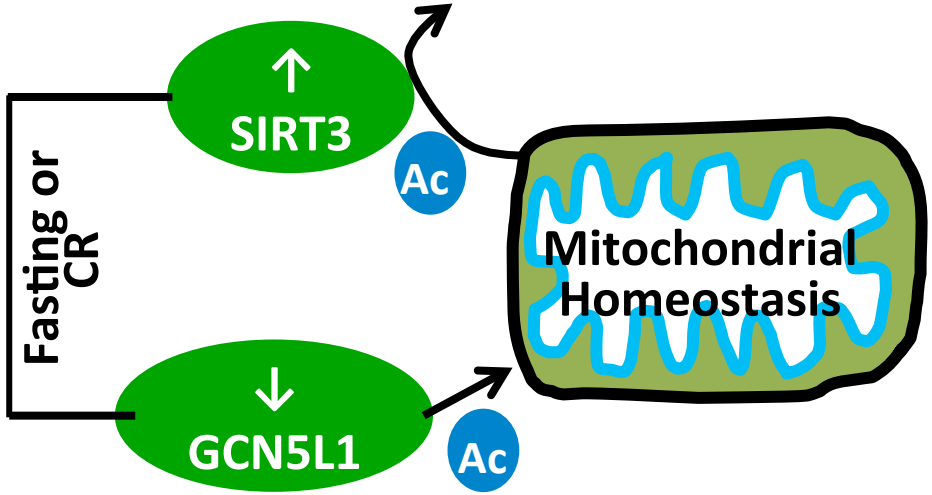
- The NLRP3 inflammasome is blunted by fasting
- NLRP3 inflammasome activation is nutrient-level dependent and appears to be modulated, in part, by the Sirt3 -Gcn5L1 regulatory program
- Preliminary data suggest that that fasting and the mitochondrial acetylation regulatory program modifies the role of mitochondria as a DAMP in NLRP3 activation
- This nutrient-sensing program is operational in healthy human subjects

hot off the press

SIRT3 deacetylase: the Jekyll and Hyde sirtuin

Dafne M. Silberman & Raul Mostoslavsky





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Brad Webster

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Richard Siegel

NHLBI

Marjan Gucek
Lance Pohl

NIH National Heart, Lung, and Blood Institute

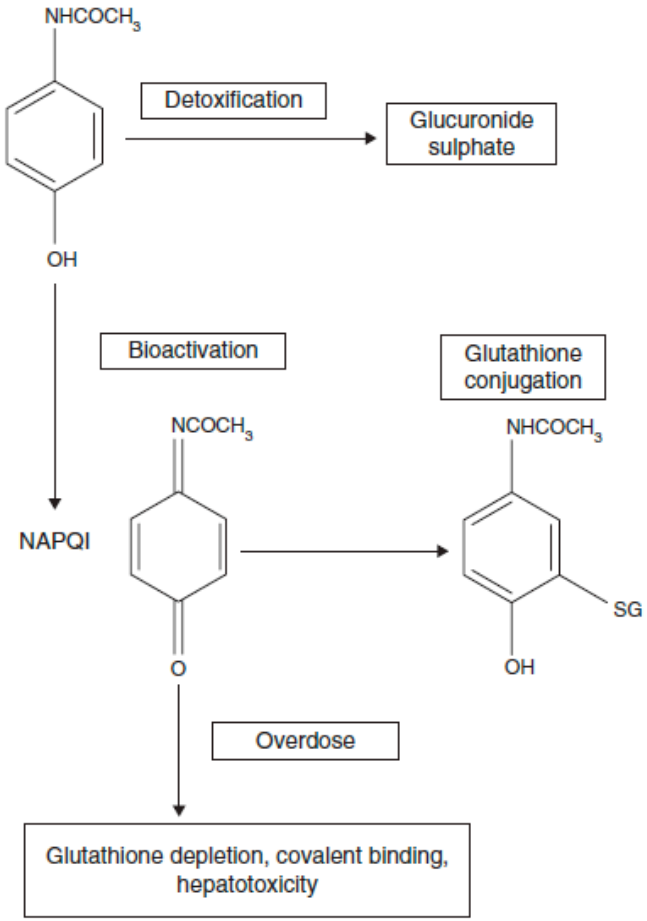

Figure 1. Metabolic activation of APAP. APAP is primarily detoxified by glucuronidation and sulfation in the liver. APAP can also undergo conversion to the chemically reactive species NAPQI by cytochrome P450. NAPQI can undergo biological inactivation through GSH conjugation but when GSH stores are depleted free NAPQI can oxidise and covalently modify proteins resulting in hepatotoxicity. The toxicological and pharmacological properties of the molecule are a function of the redox potential of the molecule.

APAP: Acetaminophen; GSH: Glutathione; NAPQI: *N*-acetyl-*p*-benzoquinoneimine.

Expert Opin. Drug Metab. Toxicol. (2008) ***4(11)***

NAPQI adducts covalently bind to cysteine residues on mitochondrial proteins contributing to hepatic toxicity

NAPQI adducts increase
The mass of a peptide by 149Da